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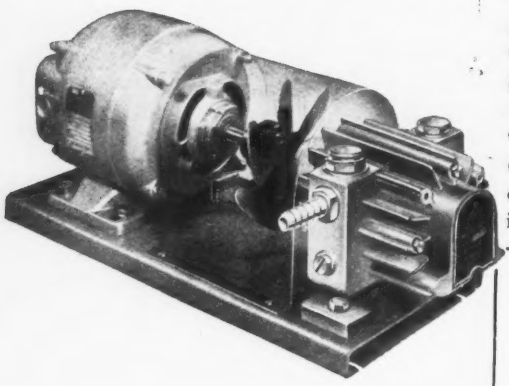

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NATURE

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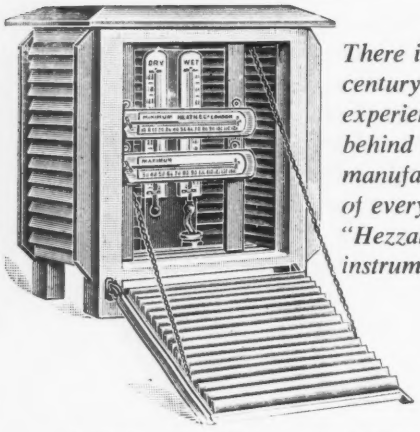

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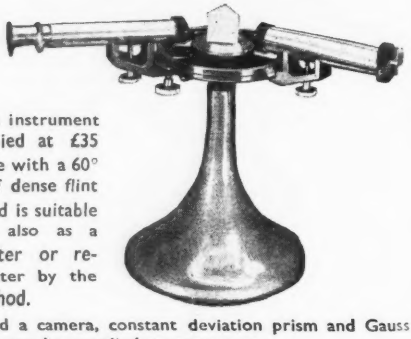

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GENERAL EDUCATION IN A MODERN DEMOCRACY

IN his presidential address to the British Association at York on September 2, Sir James Gray pleaded strongly for a wider outlook in the teaching of science and stressed the need for a considered judgment as to the proportion of our total educational effort which should be devoted to the training of scientists and technicians—upon whom we depend for maintaining or extending our standard of living—and the proportion which should be expended on raising the intellectual standards whereby the bulk of the population forms its judgments on matters which are susceptible to personal prejudice or political propaganda. Sir James recognized the implications of Dr. Trenaman's inquiry into the impact of the mass media and maintained that the key to the problem lies in the schools. The responsibility resting on secondary school teachers is not easily exaggerated, and Sir James pointed out that really inspired teachers, working with adequate but simple equipment, would achieve far more for general education than specialists in highly equipped laboratories.

In the concluding part of his address, Sir James Gray referred to the possible contribution which the British Association might make to the problem of general education so far as science is concerned, and the attention given to this question was a feature of the York meeting. Apart from this, no fewer than three of the presidential addresses to Sections of the Association discussed general or particular aspects of the main problem raised by Sir James. A provocative address by Prof. J. Jewkes to Section F (Economics), for example, examined the question of balance between general education of the population in science and that devoted to the training of scientists and technologists. In Section L (Education), Sir James Robertson raised much the same issue in frankly discussing the purpose of our educational effort, while, addressing the Assembly of Corresponding Societies, the Countess of Albemarle discussed the question how the ordinary citizen could prepare himself or herself to live intelligently in a scientific age, and the sources of information and instruction available for that purpose.

The Countess of Albemarle suggested that there are three essential needs for the ordinary citizen: awareness of the character of the times in which he lives; some understanding of the methods of scientific research; and some readiness or determination to keep abreast of scientific development and invention. As Sir James Gray had already pointed out—and as Dr. Trenaman's inquiry shows—the attempt to meet these needs must begin in the schools, and there were other papers read at York which were equally concerned with the way in which this should be done. Mr. J. Maitland, in Section J (Psychology), considered specifically the communication of science to the layman, Mr. N. F. Newbury dealt with the place of science in the primary school before Section L and, before the same Section, Dr. K. Laybourn, in discussing the training of teachers of science and mathematics and what the schools require, laid his own emphasis on the importance of quality and the decisive contribution which an outstanding or inspired teacher in love with his subject and his job could make.

It is, however, instructive to examine some of these fundamental issues debated at York in the light of the Clayton Memorial Lecture which Sir John Wolfenden delivered to the Manchester Literary and Philosophical Society in 1958. He did not attempt a definition of education, but pointed out that it is an essential part of a teacher's duty to instruct his pupils in certain areas of human knowledge and skill. This responsibility for instruction is inescapable, and it is obvious that such instruction is what is in the minds of those who raised these issues at York. Sir John, however, emphasized that education is, among other things, the influence of one personality on another. That has been clearly recognized in the great schools and universities of Britain. It is implicit in the comments made at York as to the importance and influence of the great and inspiring teacher.

Sir John Wolfenden in this lecture proceeded to discuss some of the implications arising from this influence of the teacher upon immature minds, and much of what he said is important not only in the present context but also in that of the proposal recently advanced for giving votes to young people at eighteen years of age. He faces frankly the difficulty of presenting truth objectively, particularly in relation to judgments of value, and no less he recognizes that the immaturity of the minds of our pupils is a fundamental difficulty. Because of that immaturity, they are not capable of making up their minds for themselves when confronted with carefully balanced lists of credits and debits, and Sir John believes that of all the demands made on intelligent boys and girls nowadays, this is the one which causes the most unhappiness and strain. It is not helpful to withdraw our experience and comparative maturity from them at this point.

Nor does Sir John believe that, in fact, it is possible to do so. There is no neutrality in many such matters, and there is force in his suggestion that it may be as dishonest to pretend not to hold views which one does hold as to pretend to hold views one does not. Moreover, the significant influence on the young is what a man is and does, not what he says, and what he is and does are inseparately fused with what he believes, with his convictions, principles and faith. Tolerance and an open mind are indeed essential to human thought and progress, but they must be understood and practised as positives, not as negatives. It is no part of real tolerance, Sir John reminds us, to believe that all opinions are equally

valid, or of a genuinely open mind to be empty. Empty minds and closed minds are equally a reproach to the educator, and his final plea is not for preferential treatment for any one set of opinions or doctrines, but for a free, fair chance for all, accepting the risks involved where immature minds are concerned, because in this way we come nearest to being faithful to our immature pupils and to the truth itself.

Sir James Gray suggested that the value of an educational system can be judged by the extent to which it leaves people with a desire to know more about the world at large, and aware of the need—and the possibility—of satisfying this at least in part, by personal effort. Obviously, too, a constantly changing environment involves a continuous review of the general pattern of teaching; but apart from this, it is a serious indictment of specialist training in science and technology that it so often leaves the specialist not merely with no knowledge of the humanities but also with no desire to attempt to make good the loss. That of itself has not only destroyed the catholicity of intellect without which civilization cannot survive; but it has also contributed to make the scientist and the technologist the tools, first of commercial power, and later of the impersonal power of the State.

This argument is developed by R. T. Rolt in an appreciation of the width of knowledge of I. K. Brunel with which he concludes his biography of that distinguished and versatile engineer, and his argument found echoes in a recent address of Sir Solly Zuckerman on science and freedom, as well as in Sir Charles Snow's thesis of the two cultures. The divorce of the scientist and the engineer from the humanities involves a loss of proportion, and from that springs in some measure their loss of influence, while the door is opened to misunderstandings, doubts and fears which have both shaken man's confidence and weakened his control over events. Sir James Gray wisely remarked that no young scientist should be allowed to forget that new discoveries tend to arise from the borderland between different subjects, where the discipline of one is applied to another; but that alone will not suffice. It would seem to follow from Dr. Trenaman's study that this question of general education must be tackled in the schools, before specialization begins, if anything effective is to be done.

Sir James Gray's address, no less than Sir John Wolfenden's, thus leads directly to the central question which Sir James Robertson discussed: What are our schools for? It may still be a more open question than is often admitted, how much of our educational effort should be devoted to the training of professional scientists and technologists, and neither Sir James Gray nor Prof. Jewkes denied that we might need to increase the present proportion. What they did emphasize was that we should have due regard to the cost, and before we denied, in consequence, to a very much larger fraction of the community a reasonable chance of "seeing life steadily and as a whole", we should be sure, on the basis of impartial inquiry, that the need existed and that the cost is justified.

This question, however, cannot be entirely separated from that of the general education of the community. If science is to be of direct cultural significance, it cannot, as Sir James Gray said, shut itself off from one of the main factors which have influenced men's attitude to social problems, and implicit in his address was a suggestion for further inquiry into the way in which scientists might receive this general education, possibly through the activities of the British Association itself. If this inquiry supported a wider, and perhaps more biological outlook on general education, very far-reaching re-organization of both schools and universities might be involved; but this could scarcely be undertaken without giving a considered answer to the question, what are our schools for, if not to the corresponding question about the purpose of university education. Even to urge that science can only play its full part in furthering human welfare if it is used, at a very early stage of education, as a means of encouraging a dispassionate but optimistic attitude towards all aspects of human affairs, involves at least an answer to the first question.

Sir James Robertson's survey was largely inspired by the view that we are neglecting the greater task of education for the lesser, though not unimportant, task of instruction, and it should already be clear that many of our present shortcomings arise from this fact, perhaps in part, too, from a failure to realize that what ordinary children need most of all is neither this skill nor that smattering, but just to be humanized and helped even a little way towards civilized living. It might well be urged that without this the scientist and technologist, too, are unlikely to contribute much towards meeting the real needs of mankind. If, in fact, the main effort of the secondary modern school is diverted from the general education of the ordinary child, no improvements in the extent or quality of further education are likely to repair the damage. It is abundantly clear already that the fundamental reason only a small minority of British adults continue their general education is not the lack of facilities but the defects in the education they have already received. It is in the schools and the schools alone that the basis of a sound general education can be provided, either for the ordinary citizen or for the specialist.

The whole trend of Prof. Jewkes's address supports Sir James Robertson's conclusion that until we are content to accept children as they are, we will continue to lose the chance of developing the gifts they have, in our perverse determination to make them manifest powers which in fact they do not possess. His second conclusion is endorsed by Sir James Gray's address: in its aims and emphasis our education should reflect, as it does not at present, the inescapable truth that it is for most of us relatively easy to get a job and do it decently, but tragically hard to be good human beings in our communities, our homes and our solitariness. It will not be easy to change the emphasis from vocational training to

education for life, nor perhaps even possible without a change of heart; but until it is done we can scarcely approach the problem of living with science and the effective use of the spoken or the written word and the visual image.

To set Sir James Robertson's plea that the ordinary child needs to be humanized and to be helped towards civilized living against Sir John Wolfenden's plea that it is not helpful to withdraw our experience and comparative maturity from our pupils when they are faced with judgments of value, sufficiently illustrates how much common ground exists in these two approaches and how much depends on the teacher. That remains the key problem, and especially how men and women of the right type are to be found and produced. Even if the need for Britain to produce more highly qualified scientists and technologists is fully established, the development of general education for the ordinary citizen may still remain the more urgent problem for at least two reasons. The production of scientists and technologists of the required qualities is linked with this problem of general education in the schools and the supply of appropriate teachers. Moreover, since the general citizen, as the Countess of Albemarle observed, needs increasingly to have some understanding of what science is about, because decisions in public affairs nowadays usually in some measure and at some point involve an assessment of scientific and technical data, the appropriate measures to increase the supply of teachers—and in turn of scientists and technologists—depend very largely on the existence of an informed opinion capable of understanding the action required, and capable of supporting it until it has been carried to a satisfactory conclusion.

RESEARCH IN GAS DYNAMICS

Fundamentals of Gas Dynamics

Edited by Howard W. Emmons. (High Speed Aerodynamics and Jet Propulsion, Vol. 3.) Pp. xiii+749. (London: Oxford University Press, 1958.) 140*s*. net.

THE third volume in the Princeton series, "High Speed Aerodynamics and Jet Propulsion", is concerned with the fundamentals of gas dynamics. In his preface the editor of the volume describes gas dynamics as a rapidly developing branch of physics and applied mathematics. Tsien's introductory chapter, a general development of the equations of gas dynamics but dealing principally with the fluid mechanics of a continuum, amply illustrates the importance of applied mathematics in this field of study. All possible combinations of compressible flows, adiabatic or diabatic, irrotational or rotational, steady or unsteady, are considered. The last chapter on the flow of rarefied gases, by Schaaf and Chambré, defines clearly the regimes of gas dynamics, develops equations for free-molecule and slip flows and presents experimental data for the slip flow and transition regimes. This chapter emphasizes that a broad knowledge of physics is required by a worker in the field of high-speed aerodynamics.

Yet any engineer concerned with problems in gas dynamics will question whether Emmons's definition is complete, for while the need for a background in mathematics and physics will be agreed, the development of any branch of fluid mechanics as complex as this one must depend upon verification of analytical work in engineering experiments. Crocco's chapter on one-dimensional flow (a 'book' of three hundred pages in itself) supplies a logical analytical development but shows an awareness of this need for experimental data. It is surprising that some of the now classical descriptions of one-dimensional compressible flows (for example, the flows in ducts with friction and heat transfer under varying pressure ratios) have not been backed by a great deal of experimental work. But Crocco has gathered together some excellent photographs of shock phenomena and presents the results of experiments that this reviewer has not seen before, particularly the work of Frössel. Some interesting experimental data obtained by Neumann and Lustwerk for the 'pseudo-shock' (the complex oblique shock wave pattern with turbulent mixing, which occurs when the boundary layers are thick) are also included, together with a review of the work of Shapiro and his associates on the 'aerothermopressor', a device in which the stagnation pressure of a compressible fluid flow may be increased due to the abstraction of heat (by injection and evaporation of water droplets).

Hayes's chapter on shock waves and gas dynamic discontinuities and that on shock wave interaction by Polachek and Seeger are well written and logically developed. (This latter chapter contains some beautiful photographs of regular and Mach reflexions and intersections and of shock refractions.) Stever's article on condensation phenomena, again interesting and readable, appears somewhat out of place in a volume in this series, for much of the material deals with steam flows. Kantrowitz's chapter on unsteady gas dynamics appears to be a little brief to this reader, a non-specialist in the field, who found the brevity of the last article on the application of pressure waves in heat engines disappointing, especially as the quoted references on this subject are difficult to obtain. (There are numerous references elsewhere in the volume to papers produced 'internally' by laboratories. This growing practice of referring to unpublished work is to be deplored.) Emmons's own individual contribution to the book, on flow discontinuities associated with combustion, is a lucid piece of writing, although the difference in notation between this section and the introduction on general aspects of combustion, by von Kármán, is a little confusing. Sir Geoffrey Taylor, with assistance from R. S. Tankin, provides a section on the Chapman–Jouguet theory of detonation.

One surprising omission is the lack of a chapter on the flow of real gases at high temperatures (that is to say, dissociated or ionized gases), although Crocco includes articles on the flow of gases with variable specific heats and gases obeying van der Waals's equation.

Most workers in the field of gas dynamics will wish to know how this book compares with Shapiro's volumes on compressible flow, published in 1953, but the comparison is a difficult one to make for Shapiro's book is largely devoted to the teaching of gas dynamics. The present volume is aimed at reviewing the state of research in gas dynamics, and will be widely used by specialist research workers in different fields.

If any criticism may be made of the new volume, it is the usual criticism of a book by several authors —that of duplication of content material and differ-

ing notation. (For example, the work by Hawthorne and Cohen, on the effect of heating and cooling a compressible flow, appears in three places.) In general the book is of the high calibre expected of the distinguished authors and editor, and all physicists, mathematicians and engineers working in the field will be grateful for the publication of this volume.

J. H. Horlock

MICROSCOPES AND PHOTOMICROGRAPHY

The Practical Use of the Microscope

Including Photomicrography. By George Herbert Needham. Pp. xxiv+493. (Springfield, Ill.: Charles C. Thomas; Oxford: Blackwell Scientific Publications, 1958.) 117*s*. 6*d*.

Photomicrography

By Dr. Roy M. Allen. Second edition. Pp. xiii+441 (54 plates). (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1958.) 67*s*. 6*d*.

Applied Microscopy and Photomicrography

By H. M. Malies. Pp. 143+12 plates. (London: Fountain Press, 1959.) 22*s*. 6*d*. net.

In many branches of scientific work the need to take photomicrographs arises both for record purposes and to illustrate lectures and published works. It is generally accepted that success in photomicrography primarily depends upon a thorough knowledge of microscopical principles and the correct use of the microscope: it is with this that the book by G. H. Needham is more concerned. From a study of the list of contents, it would seem that in scope and breadth of treatment this would be a most valuable book; unfortunately this does not prove to be the case, for here is one book which definitely cannot be recommended. The treatment is unjustifiably dogmatic, and much of the matter is obsolete, for example, the chapter on electron microscopy, which only makes a passing reference to the technique of thin-sectioning. Other valuable modern techniques such as phase-contrast and interference microscopy receive only a sketchy treatment.

Although the book is profusely illustrated, it is felt that many of the photographs, particularly those of microscopes, are superfluous. The essential features in a chapter on binocular microscopes, for example, are certainly not photographs of the stands, but diagrams which give the reader valuable information on, say, the arrangement of the prism trains and path of the light rays. It is these features which are conspicuous by their absence. The book has not even the merit of correct English or clear explanation to commend it. The glossary of microscopical terms included in the book may be taken as representative of the general standard: thus it is stated that "there is no connection between the focal length of an objective and its working distance, except that they are directly proportional"! It seems that there is still need for a comprehensive reference work on microscopy.

The other two books may be recommended without reserve. Dr. Allen's well-known "Photomicrography" appears in a revised and enlarged edition which gives a straightforward account of the principles and practice of photomicrography, including a chapter on home-made equipment which should prove useful to the enthusiast who has not the resources of a fully equipped laboratory at his disposal. There is also an adequate section on the purely photographic aspects which should prove valuable to many. The book by H. M. Malies has the great advantage of a low price, but it may be said at once that one gets very good value for money as it gives a clear account of basic microscopical technique. There are many good line illustrations and plates. A minor shortcoming is the lack of detailed information on the photographic processes involved in the making of a photomicrograph; I would have liked to see a section on the use of the 35-mm. camera included, in view of the increasing popularity of this size of negative for much of the routine photomicrography carried out to-day.

S. Bradbury

LOW-TEMPERATURE ENGINEERING

Cryogenic Engineering

By Russell B. Scott. Pp. xi+368. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1959.) 5.60 dollars; 42*s*.

The word 'cryogenic' has come into fashion during the past five years and is intended to embrace all practical applications of low temperatures (say below − 150° C.). In fact, it connotes, at the present time, a more restricted field in which the main emphasis is on techniques for the liquefaction and handling of low-temperature rocket fuels and oxidants, and special apparatus for low-temperature research. The main fields of industrial low-temperature application, namely, air separation and ethylene production, remain largely outside its orbit. This emphasis is clear in Dr. Scott's book and arises naturally from the fact that the work of the U.S. Bureau of Standards Cryogenic Laboratory at Boulder, Colorado, of which he is head, has concentrated on the new applications of very low temperatures.

The book is exceptionally valuable because the author combines a masterly understanding of the theory of his subject with access to detailed information on the most modern techniques, which he freely imparts.

The chapter on gas liquefaction is useful because of its comprehensiveness and its detailed treatment of heat-exchanger design. Hydrogen and helium liquefaction feature largely. Gas separation is intentionally given only token reference, but this is compensated for by a useful treatment of gas purification by adsorption.

Low-temperature thermometry is covered comprehensively and lucidly, as one might expect from an exponent of the subject.

The storage and piping of liquefied gases is discussed in great detail, both theoretically and practically. It is probable that the material given will be adopted as the standard text for many years to come.

The final chapters of the book contain a compilation of the properties of cryogenic fluids and structural materials at low temperatures, gathered and sifted with the full resources of the National Bureau of Standards.

The book is well written, and contains an ample bibliography.

G. G. Haselden

A NEW INTRODUCTION TO GEOLOGY

Basic Geology for Science and Engineering
By Prof. E. C. Dapples. Pp. ix+609. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1959.) 76*s*. net.

"BASIC Geology for Science and Engineering" is designed to introduce geology to those studying to become geologists, agronomists, or engineers. The scope is wide; except for palæontology, all branches of geology are touched on, although physical geology is given greatest attention. More prominence than is usual in a book on general geological principles is given to the soil, a topic which is introduced early in the book and returned to repeatedly in different contexts. Indeed, the arrangement of subject-matter throughout is cyclical in character, return being made to different aspects of the same point as knowledge of contiguous subjects is built up. This system is intended to facilitate the assimilation of some of the more difficult ideas. It is not altogether certain that it succeeds, although it sounds logical, but it is certain that the arrangement is not conducive to rapid reference. This is a pity, because the volume contains an impressive amount of up-to-date information, much of which has not found its way into a geological text-book before, and which often seems likely to be of greater interest to the advanced student or even the specialist in need of a quick reminder than to the beginner to whom the book is primarily addressed.

At the present time geological research is tending to develop an increasing quantitative bias. "Basic Geology for Science and Engineering" will appeal to those who believe that this is an inevitable and desirable change. The approach to the subject is essentially analytical, and frequent recourse is made to empirical relationships and the theories and principles of physical chemistry and thermodynamics in introducing and explaining geological processes. This approach has the merit that it uses points of view and processes of thought familiar to those whose interest in geology is a by-product of a more fundamental interest in some branch of engineering or other technology.

There are those, however, who consider that the variables involved in geological processes are so many and so little understood that we are very far from being in a position to rationalize the subject along rigid mathematical lines—if it will ever be possible to do so. Without taking the extremist view that all attempts to introduce quantitative concepts into geology are therefore a waste of time if not actually subversive, there does seem to be a danger that the newcomer to geology may be left with a misleading impression of simplicity, order, and exactitude if he is introduced to the subject by means of a series of mathematical equations. Prof. Dapples himself mentions the dangers of over-simplification, but he might well have given this point more emphasis. One way of doing so would have been to stress the importance of work in the field, an aspect of geology which receives scant attention. As a distinguished British geologist is fond of impressing on his students, the best geologist is the one who has seen the most rocks, and there is no doubt that familiarity with rocks, especially in the field, induces a healthily corrective scepticism towards glib generalizations, however elegant.

So long as the limitations of the mathematical approach are borne in mind, "Basic Geology for Science and Engineering" will fulfil a useful purpose, but as an introductory text-book it will probably be of greater value to engineers than to those whose primary interest is geology.

The reading lists are compiled almost exclusively from North American sources.

J. C. M. TAYLOR

DIELECTRICS

Progress in Dielectrics
Vol. 1. Edited by Dr. J. B. Birks and Dr. J. H. Schulman. Pp. x + 312. (London: Heywood and Co., Ltd., 1959.) 70*s*. net.

THIS book is the first volume of a series which is intended to appear annually. The present volume contains four reviews which cover fairly completely the recent advances concerning electric breakdown, two articles on ceramics and one on electrophoresis. The editors appear to aim at the experimentalist with theoretical interests.

The first article, on "Dielectric Breakdown in Solid Insulation", by J. H. Mason, brings up to date a subject last summarized by S. Whitehead in 1951. The author assumes a knowledge of Whitehead's book and concentrates on recent work, in much of which he has played an active part. The review is comprehensive and includes very recent work on electromechanical breakdown in plastics and thermal impulse breakdown in glass. For the electrical engineer there is much practical information regarding breakdown by internal and surface discharges and electrochemical deterioration.

"Dielectric Breakdown Effects in Crystals" is very much the preserve of its reviewer, J. W. Davisson. The subject is essentially academic and involves both advanced theory and refined experimental techniques. Davisson reviews recent work since Whitehead's book, and is careful to state fairly the conflicting views on controversial subjects, such as the theories of path formation.

"The Electric Strength and High Field-Conductivity of Dielectric Liquids", reviewed by T. J. Lewis, is a very difficult subject, because much of the experimental material is contradictory. The author, who has great experience in the field, does his best to explain the reasons for the discrepancies, but even so, the reader is left a little bewildered by the many sources of experimental error and the many alternative theories. However, just in this kind of subject it is very helpful to have a critical review.

The review of "Gaseous Dielectrics" by T. W. Liao and R. E. Plump is concerned not only with electric breakdown, but also with physical and chemical properties such as heat transfer and flammability. It is a merit of this review that it treats physical and chemical considerations with equal interest, and that it brings out the general principles of its subject in an easily intelligible way. The article contains also a number of useful practical considerations.

The article on "Ferroelectricity of Barium Titanate Crystals" by A. D. Franklin is perhaps not quite as useful as the others because its subject cannot easily be separated from ferroelectricity in general, a subject which has been widely reviewed recently, both from the crystallographic and electrical point of view In the circumstances it might have been better if

the article were more chemical and technological than it is, even though there are some useful references to chemical preparation.

"Non-oxide Ceramic Dielectrics" by P. Popper would seem to contain rather too much theory which is not immediately relevant. However, it is useful to have information on this interesting new subject by an author who is actively engaged in research on it.

"Electrophoretic Deposition of Insulating Materials", reviewed by J. B. Birks, is a practical subject which involves much chemical 'feel'. The author succeeds in presenting the relevant theoretical background of his subjects clearly and concisely, which is useful since colloid science is generally treated in a biological context. The article gives a helpful survey of the practical applications.

V. Daniel

EMBRYOLOGY

A History of Embryology

By Dr. Joseph Needham. Second edition, revised with the assistance of Dr. Arthur Hughes. Pp. 304+18 plates. (Cambridge: At the University Press, 1959.) 52*s*. 6*d*. net.

THIS new edition of Dr. Needham's remarkable contribution to the history of science will be widely welcomed. Its first appeal is to professional biologists, who will (or should) want to know more about the way their own science found its way out of abysmal ignorance and superstition towards greater knowledge and understanding, and historians of science will find it an indispensable source-book.

But it is important for wider reasons. It is important for the general historian, who will find in it numerous illustrations of the social relations of science. One that I found illuminating was the fact that in the seventeenth and eighteenth centuries there was a widely held conviction (abundantly justified by later events) that research into the nature of generation would throw light on orthodox theological doctrines, such as that of 'original sin', and that this "led to an economic situation of value for biological development". To-day it is devoutly to be wished that the powers that be, including public opinion in general, would extend this conviction and realize that research in biology will throw light on the central problem of man's nature and destiny.

The historian will also find many examples of the unfortunate political and social results of wrong attitudes to science and technology; for example, the contempt of antiquity for the 'base mechanic' and his arts, and the recurrent incomprehension of science and scientific method by governments and dominant classes. As Sir Charles Snow has so pithily pointed out in his recent Rede Lecture this incomprehension between the professional scientists and the representatives and products of so-called humane studies can be mutual, and in Britain has led to the development of two cultures within the one nation.

It would seem that the only way to heal this split is through some reform of education, aimed at the integration of the sciences and the humanities in the cultural process, and many of us feel that for this the historico-evolutionary approach is necessary.

The evolutionary concept links man with the rest of life, mind with matter, contemporary history with archæology, while the history of science and its gradual invasion of new fields can be the bridge between the scientific and other elements in human history. Books like Dr. Needham's are of the greatest value in helping to realize this process of our cultural re-education and re-integration.

Julian Huxley

FOURIER SERIES

Trigonometric Series

By Prof. A. Zygmund. Second edition. Vol. 1: pp. xii+383. Vol. 2: pp. vii+354. (Cambridge: At the University Press, 1959.) 84*s*. net each volume.

ZYGMUND'S authoritative treatise, which first appeared as a single volume in 1935, has been thoroughly revised and much enlarged for this second edition. The first volume contains practically everything which was in the original edition. The essential foundations on convergence and summability are dealt with in the earlier chapters; the reader needs a firm grasp of the elements of point-set theory and of Lebesgue integration. The main results are illustrated in a good chapter on special Fourier series. The rest of the first and the whole of the second volume deal with special problems and topics, much of the material in the second volume being work done during the past thirty years, showing, in particular, the influence of Littlewood and Paley. Each chapter is closely packed, and only the very indolent will ignore the additional wealth of content available in the annotated exercises.

The author marshals his material skilfully. A good example is his chapter on interpolation of linear operations, where the Riesz–Thorin interpolation theorem and the famous Riesz–Fischer, Hausdorff–Young and Riesz theorems which interpret and generalize the Parseval formula:

$$\frac{1}{2\pi}\int_0^{2\pi} |f|^2 dt = \Sigma |c_n|^2$$

for a function f with Fourier coefficients c_n, are neatly stitched together and embroidered with Paley's remarkable theorem on Fourier coefficients and the Hardy–Littlewood theorems on re-arrangement of Fourier coefficients. His chapter on multiple Fourier series emphasizes the need for significant rather than obvious generalizations.

Even where the ground has been well ploughed, some problems remain. For example, it is now more than eighty years since du Bois-Reymond constructed a continuous function with a Fourier series diverging at one point; the extension to divergence at an everywhere-dense set of points followed easily enough, but so far all such sets have been of zero measure. The question still stands: Can a continuous function have a Fourier series which diverges at all points of a set of positive measure? A similar problem was solved some thirty years ago by Kolmogorov, with a delicate argument producing an integrable function with a Fourier series diverging everywhere.

In its new form, beautifully produced by the Cambridge University Press, this book remains the standard and indispensable text for any analyst interested in Fourier series for their own fascinating sake.

T. A. A. Broadbent

Beiträge zur Neotropischen Fauna

Herausgegeben von Prof. Dr. Erich Titschack und Dr. Hans-Wilhelm Koepcke. 1 Band, Heft 3: Zur Kenntnis der Pseudoscorpioniden-Fauna des Andengebietes. Von Max Beier. Pp. 185–228. Kritische Untersuchungen der Newportia-Arten. Von Wolfgang Bücherl. Pp. 229–242. Ein neuer *Asthenes* (Aves, Furnariidae) von der Küste und dem westlichen Andenabhang Südperus. Von Maria Koepcke. Pp. 243–248. Beiträge zur Kenntnis der Fische Perus II. Von Hans-Wilhelm Koepcke. Pp. 249–268. (Jena: Gustav Fischer Verlag, 1959.) 10.95 D.M.

THE first two papers in the above collection which continues this important new publication are of considerable interest to students of the multitudinous neotropical invertebrate fauna. Dr. Beier deals with a rich collection of pseudoscorpions from isolated regions of the Andes, especially Peru. This region appears to be the developmental centre of genera like *Stenolpium* and *Parawithius*, while in Chile the nearctic genera like *Dinocheirus* fade out. There are many endemic species here, and Dr. Beier has found it necessary to make several new genera, all of which appear to be soundly based. Whether the Pseudoscorpions show replacement of species by altitude is difficult to determine: this is shown by such animals as some of the Chilopods, but unfortunately few of Beier's specimens have altitude data with them. The new genus *Teratolpium* is found at high altitudes only, but conversely *Pachyolpium granulatum* Beier is found from 700 to 1,630 metres. It is to be wished that, where a choice was available, the type specimen chosen should have been one from a known altitude (cf. *Progarypus peruanus* Beier). Problems for future investigation may be glimpsed here and there; for example, *Lamprochernes*, a genus of world-wide distribution, has a species high up in the Andes, while *Apolpium vastum* Beier has been found only on orchids. Bücherl's very important revision of the Scolopendromorph genus of Centipedes, *Newportia*, places the systematics of this group on a satisfactory basis for the first time. The three subgenera *Newportides*, *Scolopendrides* and *Newportia* had been used or not by previous authors almost according to taste or fancy. Bücherl shows quite conclusively that they apply to well-marked groups of distinct geographical range. A really workable key covers all the known species and sub-species and entails several changes of attribution and status. F. A. TURK

Crushing and Grinding

A Bibliography. Pp. ix+425. (London: H.M. Stationery Office, 1958. Published for the Department of Scientific and Industrial Research.) 35*s.* net.

THIS bibliography will be invaluable to the very many industrial users of the processes of crushing and grinding and to research workers interested in comminution, particle size determination and particle classification. The bibliography proper is preceded by short authoritative reviews on: fundamental aspects of crushing and grinding; problems of breakage and structure of coal; methods of particle size analysis; industrial grinding; crushing and grinding in the ceramic industry; grinding in the cement industry; crushing and grinding of minerals; grinding in the field of dyestuffs and organic chemicals; fire and explosion hazards in crushing and grinding operations. The sections into which the bibliography itself is divided (with the number of references in each section shown in brackets) are: fundamental aspects (450); crushing and grinding practice (354); coarse reduction (154); fine reduction (579); non-mechanical methods (63); materials (744); methods of particle size and surface area determinations (186); classification (160); dust and fire hazards (141). Nearly every reference is accompanied by a useful abstract. There is a name index and a comprehensive subject index. The Department of Scientific and Industrial Research and the small committee, under the chairmanship of Mr. A. S. White, appointed to advise on the planning of the work, together with Mr. W. H. Bickle, who undertook most of the detail of the work, are to be congratulated on this compilation. This is the second bibliography published by the Department of Scientific and Industrial Research on unit operations—the first was on industrial drying (1951); it is to be hoped that further similar bibliographies will be produced. S. G. WARD

River Pollution

1: Chemical Analysis. By Dr. Louis Klein. Pp. ix+206. (London: Butterworths Scientific Publications; New York: Academic Press, Inc., 1959.) 30*s.*; 3.50 dollars.

THIS book is an expansion and revision of two chapters which appeared in an earlier work concerned with the general aspects of river pollution. The pollution of the rivers of Britain has been widely discussed during recent years both in lay and scientific circles. While it may well be true that "one photograph, particularly if it is in colour, may be worth a hundred dissolved oxygen samples in so far as securing public support or influencing a jury is concerned", the satisfactory resolution of the many problems involved can only be achieved after recourse to analytical methods.

Dr. Klein deals in this text with physical and chemical methods for the analysis of waters, sewage and trade wastes; biochemical methods are not considered. The recent recommendations of the official "Methods of Chemical Analysis as applied to Sewage and Sewage Effluents" and the "Recommended Methods for the Analysis of Trade Effluents" prepared by the Joint Committee of the Association of British Chemical Manufacturers and the Society of Analytical Chemistry are incorporated. No attempt is made to give detailed procedures but the many methods available are discussed critically and the most suitable method for a particular problem is indicated. The bibliography contains nearly 600 references to the literature. The appendix includes tables of saturation values for dissolved oxygen, conversion tables for various units of measurement, including degrees of hardness, tables of alkali conversion factors and various volumetric factors, and some typical analyses of waters and effluents.

There seem to be few errors; one, common to many text-books, is that ferroin can be used as indicator in the dichromate-ferrous titration. Unless an unusually high acid concentration is used the end-point is poor. The book would be of greater practical importance had experimental detail been given for the more widely applied methods. However, it should certainly prove a valuable guide to the literature of this important field. R. BELCHER

Bumblebees

By Dr. John B. Free and Dr. Colin G. Butler. With two appendices by Dr. Ian H. H. Yarrow. (The New Naturalist : a Survey of British Natural History.) Pp. xiv+208+25 plates. (London : William Collins, Sons and Co., Ltd., 1959.) 25*s*. net.

IN their preface the editors express the hope that this book will widely encourage naturalists to take up the study of bumblebees, and it is indeed a stimulating work. Much information is given in an easily understood form, and possible answers to problems as yet unsolved are suggested. This eagerness to press forward on to untried ground does, however, occasionally lead the authors into making assumptions which are not entirely justified by the available experimental results. Certain over-simplifications also occur in places, as, for example, on p. 66 in the description of an experiment by Dr. Free, which was designed to test whether bumblebees entering a strange colony can be recognized by their scent alone. Reference to the original paper shows that the results were less clear-cut than stated here. Nevertheless, in assessing the overall scope and value of the book these criticisms prove to be relatively unimportant.

The development of colonies from their inception in the spring until the final hibernation of the mated young queens is described and, as in other chapters, the descriptions are illustrated by numerous original photographs. Among other topics discussed are the division of labour, collection of food, recognition of intruders in the nest, and predators and parasites. Not only are the biology and behaviour of bumblebees considered, however, for there is also an interesting chapter on their economic importance, in which their value as pollinators of crops and in plant-breeding is reviewed ; various suggestions are also put forward for increasing their numbers in farming areas.

Two appendixes by the authors on methods of collecting and rearing colonies contain much useful practical information, while a further two appendixes by Dr. Ian Yarrow give a simple key to the British species of *Bombus* and *Psithyrus* and details of their distribution.

M. Delia Allen

Foundations of Set Theory

By Prof. Abraham A. Fraenkel and Prof. Yehoshua Bar-Hillel. (Studies in Logic and the Foundations of Mathematics.) Pp. x+415. (Amsterdam : North-Holland Publishing Company, 1958.) 42 guilders ; 84*s*.

Axiomatic Set Theory

By Prof. Paul Bernays. With a Historical Introduction by Prof. Abraham A. Fraenkel. (Studies in Logic and the Foundations of Mathematics.) Pp. vii+226. (Amsterdam : North-Holland Publishing Company, 1958.) 45*s*.

THESE two books are the latest in the series of Studies in Logic and the Foundations of Mathematics, produced by the North-Holland Publishing Co. The first opens with a short chapter on the paradoxes of set theory and then proceeds to the axiomatic foundations, including the axiom of choice ; the axiom systems of von Neumann, and of Bernays and Gödel are discussed. There is a chapter on type-theoretical approaches, containing developments by Quine, Wang, Lorenzen and others, and an interesting section on set theories based on non-standard logics. Here, in particular, is a discussion of the rather obscure ideas of the Polish logicians, Leśniewski and Chwistek. About seventy pages are devoted to intuitionistic conceptions of mathematics, and the bulk of the discussion concerns, of course, the ideas of Brouwer. The final chapter is concerned with metamathematics and semantics. The bibliography, extending to fifty pages, covers comprehensively the years 1947–56, and will probably become the standard for this period.

"Axiomatic Set Theory" is, apart from the introduction by Fraenkel, largely a presentation of a modified form of the material published by Bernays over the years 1937–54 in the *Journal of Symbolic Logic*. It is a formal development and is carried out in detail in its applications to analysis, including the theory of real numbers, and to cardinal arithmetic. The book is, as the author says, "designed for a reader who has some acquaintance with problems of axiomatics and with standard methods of mathematical logic".

To the 'working mathematician' these two volumes will indicate something of the great amount of effort which, in recent decades, has been expended in the field of symbolic logic and the foundations of mathematics. The situation is still very fluid, and it appears that "the third foundational crisis that mathematics is still undergoing" is far from becoming a thing of the past.

L. S. Goddard

Elementary Statistical Physics

By Prof. C. Kittel. Pp. ix+228. (New York : John Wiley and Sons, Inc. ; London : Chapman and Hall, Ltd., 1958.) 64*s*. net.

THIS book contains a short but concentrated treatment of a wide field of theoretical physics. Part 1 (116 pages) deals with classical and quantum statistical mechanics and its relation to thermodynamics ; Part 2 (52 pages) with fluctuations, random processes in general (including the Wiener–Khinchine theorem), Brownian motion, noise and irreversible processes ; and Part 3 (46 pages) with detailed balance, kinetic and transport theory. The three parts are divided into a total of 45 sections, many of which are preceded by references to standard works and recent papers.

The exposition is usually clear and as simple as the topic permits, though there is an exception on p. 19 where the law of increasing entropy is discussed before the entropy of a non-equilibrium state is defined. The amount of application to particular problems is inevitably small in relation to basic theory. Assemblies of non-interacting particles only are considered, except for an example on a linear ferromagnet in the section on the density matrix and an appendix proving the virial theorem of Clausius. However, where possible, the author discusses both knotty points and recent developments ; as examples may be mentioned sections on the thermodynamics of magnetization and negative temperatures, respectively. Problems are given, but some of these seem to be intended more as invitations to follow up the references than as exercises on the text. For example, after no more formal definition of a Markoff process than the statement that the two-event probability function p_2 "contains all the information we need" the student is asked to prove the Smoluchowski (Chapman–Kolmogoroff) equation.

The book may be recommended for readers who wish to find out about some of the great variety of problems and methods in modern statistical physics.

G. M. Bell

MYXOMATOSIS: PRESENT POSITION AND FUTURE PROSPECTS IN GREAT BRITAIN

By Dr. C. H. ANDREWES, F.R.S.
National Institute for Medical Research, Mill Hill, London, N.W.7
AND
H. V. THOMPSON and W. MANSI
Ministry of Agriculture, Fisheries and Food

SINCE the introduction of myxomatosis into Australia and its destruction of millions of rabbits, the situation there has fundamentally changed, and with a startling rapidity. The virus has become attenuated so that more rabbits survive; and still more important, natural selection has ensured that the present population of rabbits has a greater innate resistance to the disease. As a result of these two factors, myxomatosis is no longer regarded as of great value in keeping down the rabbits in Australia. Workers in that country[1,4] have studied the changes in the virus and in the rabbits in a most imaginative and painstaking manner, and in consequence we now have a fairly clear picture of developments there.

Course of events in Britain. In Britain, the history of myxomatosis appears to be following a rather different course. This is doubtless due mainly to the fact that the effective vector here is, for the most part, the rabbit flea (*Spilopsyllus cuniculi*), whereas in Australia mosquitoes are the principal vectors and *Spilopsyllus* is not present.

When the disease first spread in Britain in 1954–55 it did not sweep over the country but was gradually distributed in a patchy manner and the natural local spread was slow but effective: so effective that, by the end of 1955, the great majority (well over nine-tenths) of the wild rabbits had been killed by the disease. Of course, pockets of susceptible rabbits escaped infection and continued to breed, as did the animals recovering from infection.

There were few reports of myxomatosis in the first months of 1956, but what may be called secondary outbreaks were soon evident and, as shown in Table 1, have since appeared in many places. Curiously enough, the only English counties having little or no secondary myxomatosis have been in the west and south-west, whereas the only Welsh counties ever reporting secondary myxomatosis have been the five on the west coast. It was possible to say, in 1956, that there were no really heavy rabbit infestations on the mainland, but during the past three years rabbits have gradually increased, so that there are now considerable populations in some areas and damage to crops is more frequently reported.

Samples of myxoma virus collected in the field have been sent to Prof. F. Fenner at the Australian National University, Canberra, since October 1953 and two of these, sent in September 1954 from Sussex, were somewhat attenuated[1]. The first evidence of extensive infection with attenuated virus was in Sherwood Forest, Nottingham, in April 1955[2], and other cases have since been found in many areas (see Table 1). The British attenuated or atypical myxoma usually produces a nodular lesion with less diffuse œdema than in typical cases. During the early stages the nodules may contain fully virulent virus, but when they shrink and form scabs they contain virus of reduced virulence. By means of the gel diffusion precipitin test[3] cases of typical and atypical myxoma may be rapidly distinguished and the stage of the infection assessed.

Table 1. Number of Counties in England and Wales with Outbreaks of Myxomatosis

Year	Virulent disease	Attenuated disease (No. of incidents)	
1956	21	5	(24)
1957	36	10	(42)
1958	47	22	(106)

The connexion between the virulent and attenuated strains is at present by no means clear. In the Sherwood Forest area myxomatosis has persisted continuously since 1954 and both typical and atypical infections have been present since 1955. In the Edenbridge area of Kent, on the other hand, there was no secondary outbreak of disease from 1954 until March 1958, despite the marked increase in the rabbit population, although isolated cases of infection were found. In 1958 the Edenbridge rabbits were greatly reduced by disease which was typically virulent for the first four months, after which the presence of atypical infection was also demonstrated.

Changes in virulence of the virus. As Fenner and Marshall have shown, the attenuation of myxoma virus in Australia is a logical consequence of the relationships existing between virus, host and vector. A rabbit bitten by a mosquito which carries a relatively avirulent virus will survive longer than one infected with a more virulent strain and will, therefore, be able to serve longer as a reservoir of infection; virus will thus be carried by more mosquitoes to other rabbits. In Australia natural selection thus tends to favour an attenuated strain. In fact, fully virulent viruses deliberately introduced into areas where less vicious ones are already prevalent will dominate the scene for only a short time[4]; the less virulent ones supplant them in a matter of months. Virus which is too attenuated, however, causes lesions which apparently cannot serve as a good source for virus: so the tendency is towards domination by strains of intermediate virulence.

As already mentioned, attenuated myxoma virus has appeared in some areas of Britain and is on the increase, but modified strains have not, as in Australia, steadily ousted the highly lethal ones. After six years, the latter seem to be at least as numerous as milder ones. This might have been predicted from the nature of the virus–flea–rabbit relationship, which presents notable differences from the virus–mosquito–rabbit system in Australia. In contrast

to the Australian situation, there is likely to be some evolutionary pressure in favour of a virulent virus. Fleas on rabbits infected with such a strain will naturally leave the rabbit when it dies and spread the highly lethal virus. Where, however, a rabbit survives, the *Spilopsyllus* may have no occasion to seek a fresh host. Even if the rabbit dies after a chronic illness, there may well be only a little virus on the flea's proboscis at that stage. On a short-term basis survival of the highly lethal virus could thus be favoured. Marshall and Fenner[5] have made suggestions on similar lines.

This, however, can scarcely be the end of the story. For, as Theobald Smith taught, a parasite which kills all its victims will soon perish for lack of fresh hosts to infect. There will thus be a counteracting long-term tendency to perpetuate a not too virulent virus. In practice there have been several instances where, at the beginning of a myxomatosis outbreak, highly lethal virus has been recovered, whereas in samples obtained later in the outbreak attenuated virus has predominated. Rabbits with attenuated virus may be ill for a long time and the virus may persist in attenuated form by exchange of infected fleas in burrows. The resultant of opposing evolutionary tendencies may well be a mixture of highly lethal and attenuated viruses existing side by side in the same locality. Such a result could be brought about if the favoured virus were one which, as regards virulence, was genetically unstable; but what will in fact be the outcome, only time and alertness of investigators can reveal.

Changes in resistance of rabbits. In Australia, a standard virus which originally killed 90 per cent of wild rabbits was only able, after the population had been exposed to seven successive epizootics, to kill 30 per cent of currently caught young ones[6]. This tremendous increase in resistance seems to be of more practical importance than any change in the virus; and infection with myxomatosis has now become a minor factor in controlling Australian rabbits despite the fact that there is usually an epizootic each summer. The British results summarized above do not suggest that any such change has occurred among our rabbits. One wonders, indeed, whether this would be expected in a population of hosts with an average life-span of about a year and with outbreaks of disease at irregular intervals which may, as around Edenbridge, be as long as four years.

Discussion. The main object of this article is to try to dispel the idea that myxomatosis here is bound to behave just as it does in Australia. There is already good evidence that it is not doing so, and possible explanations of this have been brought forward. We do not know how the virus persists between outbreaks and how it manages to re-appear after an apparent absence when rabbit numbers have materially increased. It could conceivably persist in a modified form in an immune population or in some biting arthropod; but evidence is lacking that either method is actually possible over a period of years. It could also be introduced on rabbit fleas temporarily carried on migrating birds, or on wind-dispersed, infected *Anopheles maculipennis* (= *A. atroparvus*) since these mosquitoes are known to be vectors in southern England, though of minor importance compared with fleas. Their role is believed to be rather greater in France, and if this is in fact so, myxomatosis there may have a different future from that in England. There remains the possibility of deliberate introduction by man, even though the Pests Act of 1954 made it an offence to use a rabbit infected with myxomatosis to spread disease among uninfected rabbits. Few would deny that the indiscriminate spreading of the disease is undesirable, particularly in the absence of much more knowledge about the infection and the long-term consequences of its introduction. In any event we must admit that we do not know which, if any, of the agencies discussed are adequate to account for the persistence of the disease and its re-appearance at times after a considerable absence.

The increase in rabbit damage to crops in 1959 serves as a reminder that, despite myxomatosis, the rabbit population of Britain is again rising. Much useful control has been possible by the concerted action of landholders through Rabbit Clearance Societies; there are now 370 of these, covering seven million acres of land or about 15 per cent of the agricultural area of Britain: it would be most unwise to relax such efforts. Above all, it is of tremendous importance for the future of farming in Britain that we should learn more of the natural history of myxomatosis and the factors making for changes in the virus and in the rabbits. Myxomatosis provides an unusual opportunity to study and compare the evolution of a host–parasite relationship in the contrasting environments of countries at opposite ends of the Earth.

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[2] Hudson, J. R., Thompson, Harry V., and Mansi, W., *Nature*, **176**, 783 (1955).
[3] Mansi, W., and Thomas, Valerie, *J. Comp. Path.*, **68**, 188 (1958).
[4] Fenner, F., Poole, W. E., Marshall, I. D., and Dyce, A. L., *J. Hyg., Camb.*, **55**, 192 (1957).
[5] Marshall, I. D., and Fenner, F., *J. Hyg., Camb.*, **56**, 288 (1958).
[6] Fenner, F., *Brit. Med. Bull.*, **15**, 240 (1959).

INTERNATIONAL COUNCIL FOR THE EXPLORATION OF THE SEA

SPECIAL INTERNATIONAL GEOPHYSICAL YEAR MEETING

As part of the oceanographic programme for the International Geophysical Year the onus of carrying out a Polar Front Survey in the North Atlantic Ocean was placed by the Comité Spécial de l'Année Géophysique Internationale (the body established by the International Council of Scientific Unions for the planning of International Geophysical Year operations) on the International Council for the Exploration of the Sea with the help of the International Commission for the Northwest Atlantic Fisheries. A sub-committee of the International Council for the Exploration of the Sea under the chairmanship of Dr. G. Böhnecke (Federal Republic of Germany) co-ordinated the research plans of the different countries, and forty-six research and other ships of eleven nations took part in the survey. At

a meeting held under the chairmanship of Dr. J. B. Tait (Britain) at the Council's headquarters in Copenhagen during October 1–3, 1959, 45 papers were presented dealing with the first results of the survey.

About sixty attended the meeting, and Mr. A. J. Lee and Dr. D. H. Cushing (Britain) acted as reporters.

Hydrography

The hydrographical papers were given in groups, according to geographical regions.

Barents Sea. The paper by Prof. I. Hela (Finland) described Finnish work at the beginning of the International Geophysical Year. Various sections were worked and can be compared with those of the German research ship *Poseidon* in 1927. The temperature and salinity of the Atlantic water penetrating the Barents Sea were higher in 1957 than in 1927. An increase in salinity of 0·04 per mille on the standard values of all the basic water masses was observed; surface temperatures and salinities were higher than the average values given in the atlas by Dr. Krauss. Mr. A. J. Lee showed that the volume transport of the West Spitsbergen Current was below normal taking the International Geophysical Year as a whole, and that temperatures in the south-eastern Barents Sea were subnormal. He related this state to the abnormally strong development of the Polar high-pressure system and the southward displacement of the atmospheric Arctic Front. Norwegian work in the area during various seasons was described in a paper by Mr. L. Midttun (Norway) which was read by title.

In the first of two papers on the chemistry of Barents Sea water, Dr. S. Gripenberg (Finland) found that the alkalinity/chlorinity ratio was higher in the Norwegian coastal water than in the Atlantic water or the East Spitsbergen Current, but that the reverse applied when the boron/chlorinity ratio was considered: this implies that most of the boric acid is bound up in organic complexes. In the second paper, Dr. A. Voipio (Finland) showed that different methods of analysis gave different results for the total iodine content of sea water and demonstrated how little we know about the iodine content of sea water.

Greenland and Norwegian Seas. Drs. T. I. Gorshkova and E. V. Solyankin (U.S.S.R.) showed that the deposits on the sea-bed, by differing in their chemical composition, particularly in their content of carbonates and of iron and manganese oxides, are indicators of the hydrographic conditions prevailing in the basins of these two seas. Dr. J. N. Carruthers (Britain) pointed out the pioneer use by Otto Pettersson of this technique. In a paper read by title, Dr. G. N. Zaitsev *et al.* (U.S.S.R.) have computed the water and heat budget of these seas. When the various components are summed, the difference between the heat input and output amounts to only 0·4 per cent. The authors then proceed to show the relative importance of these different components in different parts of the seas: advection of heat by currents is found to be the most important. Finally, they have computed the nutrient salt budget. Dr. A. P. Alekseev *et al.* (U.S.S.R.) described Russian hydrographic work in the southern part of the Norwegian Sea in 1958. In April the East Iceland Arctic Current was strong, blocking the inflow of Atlantic water to below average: in October the inflow was intensified. The waters near the bottom in this area were found to have a salinity of 34·87–34·88 per mille and the authors assumed that they are related to the East Iceland Arctic Current and flow from west to east. It was pointed out by Mr. O. Sælen (Norway) that these salinity values are lower than the standard values for Norwegian Sea bottom water. Dr. J. B. Tait (Britain) pointed out that the water might have been Arctic Intermediate Water. In reply to a question, Prof. J. V. Preobragenski (U.S.S.R.) said that the salinities had been determined by the Knudsen titration method. At this stage Prof. G. Dietrich (Federal Republic of Germany) stressed that the results collected during the Polar Front Survey should be sent to the International Council for the Exploration of the Sea as well as World Data Centres *A* and *B*. Mr. Sælen described Norwegian work in the same area in 1958, in March and October: an intensification of the inflow of Atlantic water in the latter month was noted, as had been reported by the Russian workers. A special feature observed was the ascent of cold water along the continental slope off Norway. Finally, Dr. J. Eggvin (Norway) presented a series of temperature, salinity and current charts for the Norwegian and Greenland Seas, and showed how the Norwegian Sea bottom water is formed in the region north of the Jan Mayen Ridge in some years and not in others, depending on the meteorological conditions, and how this bottom water flows southwards from the Greenland Sea along the foot of the Norwegian continental slope towards the Faroe–Iceland Ridge. It was further shown that the temperature of the bottom water of the Greenland Sea increased in temperature northwards from the main area where it is formed. This temperature increase is a result of mixing with Atlantic water. The positive difference between the temperature of the bottom water of the Arctic Ocean and that of the Greenland Sea can therefore be explained without, as previously, assuming a submarine ridge (1,200–1,500 m.) between Spitsbergen and the north-eastern part of Greenland. This is of interest in view of the recent work of Dr. L. Balakshin (U.S.S.R.), who by investigations on board ice-breakers has shown that the sill depth between the Greenland Sea and the Arctic Ocean exceeds 3,000 m. In discussion of this paper it was pointed out that the sinking of water to form the bottom water might set up a system of compensatory surface currents which would be of importance to the Barents Sea fisheries.

Shetland–Faroe–Iceland Region. Dr. J. B. Tait and Mr. J. H. A. Martin (Britain) had computed the volume transport through the Faroe–Shetland Channel over the period of the International Geophysical Year and found it to be high at the beginning and end but low at other times. Gulf of Gibraltar water seems to have been present in the oceanic water-mass in June 1957 and June 1958. In June 1957 the inflow was cut into two parts by Arctic Intermediate Water which was moving southwards, and in June 1958 it was similarly divided by a southerly flow of Norwegian Sea water. Overspill of cold Norwegian Sea water was noted along the Faroe–Iceland Ridge in June 1957 and March 1958 but not at other times.

On the basis of surveys made along the Faroe–Iceland Ridge during 1957–58, Dr. J. H. Steele (Britain) had come to the conclusion that overflow of very cold (0–2° C.) water is rare and unimportant, but that there is continuous overflow of a slightly warmer

(2–4° C.) water which is the product of mixing of water masses of Atlantic and Arctic types on the top of the ridge. This overflow has a geostrophic motion north-westwards along the southern side of the ridge, and it then turns southwards along the eastern side of the Reykjanes Ridge. The total flow over the ridge is calculated as being near the mean value of the Faroe–Shetland inflow. A discussion of this paper by Prof. Dietrich showed that, on about 50 per cent of the surveys of the ridge, overflow of cold (0–2° C.) water has been found. The relative importance of overflow directly across the ridge and outflow through the channel between Faroe and Faroe Bank was debated by Dr. Tait, Mr. F. Hermann (Denmark) and Dr. Carruthers.

North Atlantic Ocean. Prof. Dietrich described various stages in the evolution of the International Geophysical Year and considered the next stage: the exploitation of the observations. He suggested the preparation of an atlas of maps and sections of the North Atlantic. Using German observations, he demonstrated the existence of six water masses on the Cape Farewell–Flemish Cap section. In particular, he examined the origin of the North Atlantic deep water in the Labrador Basin and the overflow of cold water across the Iceland–Greenland Ridge, and showed how the latter could be tracked over a great distance clinging to the lower part of the continental slope and not flowing along the very bottom of the basin. He also showed how the winter surface isotherms are a guide to the circulation of the North Atlantic in that season.

A paper was given by Dr. W. Krauss (Federal Republic of Germany) showing that internal waves can be set up in the deep layers of the ocean as well as in the upper layers by the action of the wind.

From dissolved oxygen/potential temperature diagrams, Dr. L. H. N. Cooper (Britain) concluded that the water column in the Bay of Biscay consists of a layered series of resident water masses resembling a pile of plates stacked one on the other. He regarded these plates as being the result of the overspill of boluses of cold water across the Faroe–Iceland Ridge. He also demonstrated a secular change in dissolved oxygen content since 1922. It was pointed out by Prof. Dietrich that the stepwise structure described could also be explained by Dr. Cooper's earlier turbidity current theory, and that this structure had not been as yet found in other areas where very detailed hydrographic observations had been made (for example, south-west of Iceland). The dating of the climatic fluctuation which had brought about the secular change in dissolved oxygen content was discussed by Mr. Lee and Dr. Cooper.

French observations in the North Atlantic and Davis Strait were described by M. G. Peluchon (France). Charts of the currents in the region of 50° N. showed the meandering of the current as it leaves the Grand Banks area and a decrease in the meanders as the current proceeds eastwards.

Dr. J. Joseph (Federal Republic of Germany) described his work with a transparency meter combined with a thermocouple. The turbidity distribution depends on local production on one hand and advective processes and turbulence on the other. Connexions between the turbidity sections and the temperature sections could be seen. He also showed that there is no change in turbidity at the deep scattering layer. In discussion a strong case was made for fish as being the cause of this layer.

Dr. H. Weidemann (Federal Republic of Germany) described work with towed electrodes (*GEK*) between Greenland and Iceland. At a fixed station south of Iceland the records collected over a period of 30 hr. allowed the relation between wind and surface current to be investigated. The results gave a mean deflexion of current to the right of the wind of 27° and a current/wind ratio of 1·4–1·5 per cent. The difference between these values and Ekman's theoretical values can be explained by assuming that conditions were non-stationary.

Mr. F. Hermann described Danish observations in the North Atlantic in July–August 1958. West of the Reykjanes Ridge the basin was largely filled with sub-arctic mixed water, as already described by Prof. Dietrich. Cold water coming over the Iceland–Greenland Ridge was again found on the bottom in the western part of the Irminger Sea. East of the Reykjanes Ridge a bottom layer with a temperature below 3° C. was found, consisting of mixed water derived partly from overflow across the Faroe–Iceland Ridge, partly from Atlantic water and partly from sub-arctic water. This water circulated anti-clockwise around the basin and crossed the Reykjanes Ridge to flow north on its westward side and mix with the overflow across the Iceland–Greenland Ridge. In the Davis Strait water which had overflowed the Greenland–Baffin Island Ridge but which does not contribute to the North Atlantic circulation as a whole was detected.

Dr. R. A. Cox (Britain) gave an account of the work of R.R.S. *Discovery II*. A section showing the distribution of silicate along lat. 24° N. had three outstanding features: the depletion of silicate but not of phosphate at the surface in the Sargasso Sea; the high silicate content of the bottom water of Antarctic origin west of the mid-Atlantic Ridge; and the very irregular bottom topography. Work with Swallow's neutrally buoyant floats west of Portugal showed how even in the deep ocean there are great variations in current speed and direction. The outflow of Mediterranean water from the Straits of Gibraltar had also been tracked by these floats, and it was shown that a westward movement of 12 nautical miles/day could exist close to the Spanish coast, but that farther south there were large eddies. Dr. Cooper pointed out that the silicate distribution along lat. 24° N. showed that Antarctic bottom water must make a contribution to the bottom waters east of the mid-Atlantic Ridge, and Prof. T. Braarud (Norway) did not think that the silicate deficiency prevented phytoplankton production in the Sargasso Sea. The phosphate budget of the Mediterranean Sea was discussed by several speakers. Mr. Sælen then described Norwegian work carried out in collaboration with R.R.S. *Discovery II* west of Portugal. Current measurements from an anchored ship allowed the tidal streams to be analysed; they were present to the bottom (760 m.) and showed no decrease in velocity with depth, but there were indications of some differences in the direction of rotation. The residual current, however, decreased with depth.

Prof. N. Menendez (Spain) gave an account of temperature and salinity conditions along the meridian of Tarifa in August 1958. Sections were worked at different states of the tide and the distributions found could only be explained in terms of changing mixing conditions depending on the strength of the tidal streams.

Mr. L. V. Worthington and Mr. W. G. Metcalf (U.S.A.) examined the salinity/potential temperature

relationship in the North Atlantic deep water using the very precise salinity data that have now become available with the development of conductimetric techniques. A salinity/potential temperature curve for the western North Atlantic below the 4° C. potential isotherm has been established and its shape accounted for in terms of water masses. Departures from this curve in different parts of the Atlantic can be used as indicators of water movement. The outstanding features shown by this form of analysis were the formation of the newest Atlantic deep water in the Labrador Basin; the part played by the South-East Newfoundland Ridge in preventing the Antarctic bottom water from reaching the Labrador Basin; the water of the Norwegian Sea origin on the eastern slope of the mid-Atlantic Ridge; the outflow and spreading of Mediterranean water; and the fact that the western basin of the South Atlantic is the source of the deep cold water found in the rest of the Atlantic, communication to the eastern basin being through the Romanche Trench.

A paper by Mr. J. R. Lumby (U.S.A.) read by Dr. Tait showed that there were large differences in the dissolved oxygen values at comparable stations worked by American, British and Russian ships during the International Geophysical Year. Mr. Worthington said that such differences could depend to some extent on the type of water-bottle used, and on the method of standardization of the sodium thiosulphate solution used for titrating the samples.

Baltic and North Seas. In a paper on the southern Baltic Sea, Dr. A. Majewski (Poland) showed that the inflow of oceanic water had decreased since 1951–52 and that, at the end of 1958, the salinity in the Baltic basins had reached its lowest level since 1952. The year 1958 had been a cold one so far as the Baltic was concerned. Similarly, in a paper on the North Sea by Dr. J. Filarski (Poland), read by title, the winter and spring of 1958 were shown to have been cold, but by the autumn of 1958 there were positive anomalies of temperature. Dr. V. V. Betin and Prof. J. V. Preobragenski (U.S.S.R.) submitted a paper which was read by title on ice research in the Baltic during the International Geophysical Year. Aircraft were used to make synoptic surveys of ice conditions. The curves of accumulated temperature, ice extension and ice accretion so produced were found to be related.

Biology

Prof. E. Steemann Nielsen (Denmark) presided over the biological session.

Productivity. Three papers were presented. The first was by Mr. Grim Berge (Norway) on the productivity of the Norwegian Sea using, besides carbon-14 measurements, an estimate of productive capacity, derived from measurements of transparency. A continuously recording transparency meter was described. It was shown that in 1958 the quantity of production as measured by 'productive capacity' was different from that in 1954. In reply to a question as to whether there was a correlation between productive capacity and standing stock, Mr. Berge replied that there was, but that the relationship was different in different water masses. Prof. Braarud commented on the marked changes noticed from year to year and their apparent relation to hydrographic processes.

Mr. Vagn Hansen and Prof. E. Steemann Nielsen described carbon-14 measurements and chlorophyll measurements in the North Atlantic and in the Greenland Sea. Mr. Hansen showed that from Cape Farewell to Ireland greater counts of carbon-14 were obtained towards Greenland and above the Reykjanes Ridge. This was associated with greater quantities of chlorophyll. Prof. Steemann Nielsen demonstrated the relationship between carbon-14 counts and quantities of chlorophyll *a*, which was biased by the possible presence of dead chlorophyll. Mr. G. Murphy (U.S.A.) asked whether transparency might not be a better method of measuring productivity if the constants in the equation were known. Prof. Steemann Nielsen replied that this would be a good method in oceanic waters, but in coastal waters the quantity of inorganic material was high. Dr. M. Gillbricht (Federal Republic of Germany) pointed out that only one-third of the turbidity in the Irminger Sea was due to plankton. Mr. Berge said that the quantity of inorganic particles ordinarily was constant and so the variations due to production differences could be estimated.

Biophysics and Biochemistry. Dr. H. Schaefer (Federal Republic of Germany) described the distribution of amino-acids in redfish (*Sebastes*) and certain other fish for a number of stations at sea. It was shown that the variation in relative composition of certain amino-acids was much greater than that which might have been expected from studies in freshwater fish. Mr. Murphy noted that a similar result had appeared in the work on the Californian sardine.

Plankton. Dr. K. F. Wiborg (Norway) described the distribution of zooplankton in the Norwegian Sea. He noticed that the distribution of reverberation on the echo-sounders at full gain corresponded fairly well with the distribution of euphausiids and fish fry. The distribution of copepodite stages bore some relation to hydrographical conditions. In response to a question, Dr. Wiborg said that smaller fish have smaller eggs and spawn later.

Drs. E. A. Pawstiks and L. N. Grutzov (U.S.S.R.) presented a paper on the distribution of plankton in the Norwegian Sea. This distribution corresponded reasonably well with that presented by Dr. Wiborg.

Dr. J. H. Fraser (Britain) described indicator species in the Faroe–Iceland Ridge region and from the presence or absence of certain long-lived animals concluded that the International Geophysical Year was not a normal year. Mr. Lee pointed out that during the first eight months of 1958 the Polar Front lay well to the south, presumably holding back the northward flow of Atlantic water.

Dr. Gillbricht gave a detailed account of the distribution of phytoplankton, zooplankton and organic particles on a section between Newfoundland and the Azores. Counts were made from small water samples of 0·3 ml. for phytoplankton and of 5 ml. for zooplankton. By converting all quantities to total carbon and comparing these with phytoplankton, he was able to distinguish three water masses. Prof. Steemann Nielsen asked whether the organic particles were artefacts, because if the same technique is used in Danish waters many organic particles were derived from the destruction of phytoplankton. Dr. H. Einarsson (Iceland) noticed that the quantity of zooplankton collected with Hardy indicators in the Irminger Sea was roughly similar to that

collected with a 5-ml. water sample in the same area.

Mr. J. Corlett (Britain) described the zooplankton collected at weather stations *I* and *J* and showed that the total quantities were greater in 1958 than in 1957. Mr. Hansen noticed that *Evadne nordmanni* had also been found in the Norwegian Sea. Dr. Fraser said that *Thalia democratica* found by the Plankton Expedition in 1888 off west Scottish coasts appeared for the first time off west Scottish coasts in 1958.

Dr. W. Höhnck (Federal Republic of Germany) presented an interesting paper on the quantity and types of fungi in the sea and on the sea bed. The majority of samples taken showed development of hyphæ.

Fisheries. Dr. Eggvin presented a paper by Mr. L. Midttun on echo-surveys in the Barents Sea. In general, there was a relationship between the distribution of echo-traces and isotherms in the Barents Sea.

Conclusions. There appeared to be two main conclusions from the biological papers:

(1) The International Geophysical Year differed in two respects from some other years, in indicator species and in quantity of living material.

(2) Three advances in productivity studies were revealed: (*a*) the use of transparency as an index of productivity under certain limited conditions; (*b*) the fairly close relationship between standing stock (as chlorophyll) and productivity (as carbon-14 count); (*c*) the use of very small samples of phytoplankton (0·3 ml.) and zooplankton (5 ml.) to give sensible estimates of carbon in living material.

A. J. Lee
D. H. Cushing

EFFECTS OF FOREST AREAS ON WATER RESOURCES, AND THE TECHNIQUE OF LYSIMETRY

BETWEEN September 8 and 13, two symposia were held in Germany by the International Association of Scientific Hydrology, at Hannoversch-Münden, where the Forestry School of the University of Göttingen is established.

One symposium concerned the influence of wooded areas on the elements of the water balance. Thirty-five papers were presented, ten from the U.S.S.R., eight from the United States, four from Great Britain, two each from Finland and Poland and one each from the Belgian Congo, Czechoslovakia, Denmark, French Africa, Germany, Holland, Hungary, South Africa and Switzerland.

The other symposium dealt with the technique of lysimetry and the causes of error in results obtained. There were seventeen papers, four from the United States, three from Holland, two each from the Belgian Congo, Germany and the U.S.S.R., and one each from Austria, France, Hungary and Great Britain.

The papers were made available in printed form at the meeting and have since been placed on sale by the Association*. Most of them are written in English, and the few others in French or German.

The symposia were attended by more than a hundred hydrologists, from other countries as well as from those which contributed papers. There was naturally a strong German representation, while both the United States and Great Britain had important teams. It was regretted that, while the U.S.S.R. had sent several valuable papers, their authors were not present to introduce them.

Below is given an appreciation of each of the two symposia and of a two-day visit paid afterwards to German field-stations concerned with one or other of the two subjects that had been discussed.

It is expected that the discussions of the papers will be reported briefly in the quarterly issues of the Association's *Bulletin*, the price of which is 150 Belgian francs yearly.

* Publication No. 48 (Vol. 1: Water and Woodlands). Pp. 340. 300 Belgian francs. Publication No. 49 (Vol. 2: Lysimeters). Pp. 169. 150 Belgian francs. Obtainable from Mr. Arthur F. Bird, 66 Chandos Place, London, W.C.2, or the Secretary of the International Association of Scientific Hydrology, Prof. L. J. Tison, 61 Rue des Ronces, Gentbrugge, Belgium.

Water and Woodlands

In many countries, increasing concern with the provision and maintenance of adequate water supplies in the face of continuously increasing demands has stimulated considerable interest in the scientific management of this most vital of our natural resources. With fuller appreciation of the importance of form of land use in catchment areas, much attention has naturally been directed to the role of a forest cover. Compared with other countries such as the United States and Germany, Britain is a rather late entrant into this field, but within recent years, the problem has come very much to the fore and there can be no doubt, especially in view of the recent drought, that we shall have to devote very much more attention to this important issue. As elsewhere, differences of opinion exist as to whether, from the hydrological point of view, our catchments are better under forest than, say, under pasture. The answer is by no means as clear-cut as some would make out; the hydrological relationships involved are most complicated and objective quantitative assessments beset with considerable practical difficulties. It was therefore most timely that under the auspices of the International Association of Scientific Hydrology much of the experience and present knowledge in this field could be surveyed and discussed.

Almost half the contributions were concerned with investigations on the catchment scale. In principle, these involve the measurement of precipitation and run-off (both surface and subsoil), normally by stream gauging; despite the substantial cost of installation and maintenance, and, very often, difficulties in ensuring absence of leaks and a reasonable standard of precision, this approach is still essential for the provision of the basic hydrological data appropriate to the problem as a whole. As an alternative to the 'straightforward' comparison, say, between forested and non-forested catchments, Idson (U.S.S.R.) preferred continuous measurements on an area under the influence of a varying forest cover. Because of the well-known difficulties in ensuring comparability between catchments, this

latter approach would generally seem to be the more reliable one; the regression techniques used by Idson and by Anderson and Hobba (U.S.A.), in which run-off is related to the various meteorological, soil or land-use factors which influence run-off, offer an approach which allows for a more complete interpretation of the complex relationships involved.

In those countries where snow forms an important source of water there was general agreement as to the beneficial influence of a forest cover; the data of Anderson and Hobba (U.S.A.), Goodell (U.S.A.), Martinelli (U.S.A.) and Sozykin (U.S.S.R.) clearly showed that through accumulation and the shelter provided, the forest retards thawing, reduces the danger of spring floods and prolongs the supply of melt water. The difficulties of measuring snowfall and the need for further investigation were made clear in the papers of Martinelli (U.S.A.) and Sepännen (Finland).

General recognition was also given to the higher permeability and greater storage capacities of soils developed under forest, leading to reduced surface run-off, less erosion and a more prolonged yield of water during drought. Anderson and Hobba (U.S.A.), Valek (Czechoslovakia) and Banky (Hungary), among others, clearly demonstrated the regulatory effect of the forest on stream flow; Rodier (French West Africa) showed that flood peaks were 8–12 times lower from forest than from savannah, and the importance of this effect was recognized by Wicht in his published recommendations for the management of catchment areas in South Africa.

So far as absolute quantities were concerned, most contributors were prepared to accept a somewhat lower yield from a forested area as compared with areas under other vegetative covers. Meginnis (U.S.A.) provided one of the more extreme examples from the classical Coweeta experiments in North Carolina, where clear cutting of mountain hardwood forest increased the annual yield by 11–17 in. and cutting of the shrubby understory by 2 in. To conserve water in the south-western United States, Horton recommended the clearance of phreatophytes (vegetation with permanent access to ground water) along streams and rivers. While such losses from forest stands were generally attributed to higher levels of transpiration, usually because of deeper rooting and access to water during dry periods, some would attempt to explain at least some of the losses to interception of precipitation by the foliage. Thus, Eidmann (Germany) stated that because it intercepts appreciably less rainfall, a beech forest conserves more water than a spruce forest. The implied assumption that intercepted water means a corresponding loss to the soil was contested by Leyton and Carlisle (Great Britain), who produced experimental evidence indicating, as might be expected, a marked fall in transpiration following wetting of the foliage; attention was also directed to the possibility of rather large errors in the estimation of through-fall in a stand using a limited number of gauges, and the increase in accuracy obtainable when these were replaced by troughs with larger collecting areas. The papers of Bochkov and certain other Russian contributors provided an interesting contrast to the generally prevailing opinion that a forest cover means a lower yield of water. These authors argued that, because of deep ground-water movement in forest soils, the gauging of small streams draining small catchments may under-estimate the yield and that over large areas, in certain cases at least, yield from the forest may be even higher than that from open land. Sokolovsky (U.S.S.R.) also claimed that an increased water loss from forest by transpiration may be balanced by reduced evaporation from the soil. It is possible, therefore, that even in the case of water yield, final judgment on the effect of a forest cover may have to be postponed; from the point of view of energy relations, at least, one would not expect large differences in the loss of water from different types of vegetative cover.

A number of contributors described other means of investigating quantitatively the hydrological relations of a site. Visser (Holland) explained his soil moisture flow approach, which provides an estimate of the water balance from measurements of rainfall, potential evaporation and the ground-water level in the soil and in ditches; Lebedev (U.S.S.R.) also provided an interesting analysis of ground-water dynamics under forest and grass covers. From measurements of water loss from detached shoots of Scots pine, Rutter (Great Britain) gave evidence of transpiration values apparently exceeding Penman's estimate of E_T (potential evaporation from grass); this, combined with his observations that the trees continued transpiring even down to a soil moisture deficit of 7 in. or more, introduces still further problems in our interpretation of forest hydrological relationships. As yet another approach to the estimation of water losses from forest stands, Leyton (Great Britain) discussed the possibility of measuring the volume rate of sap flow in tree stems by the heat-pulse method originally introduced by Huber. It was regretted that, apart from a few words in discussion by Baumgartner (Germany), there was no opportunity to learn of the present status regarding the energy-balance approach.

In summarizing one's general reaction to the papers reported above and to the subsequent discussions, one cannot but be impressed by the magnitude of the efforts made to gain a better understanding of the hydrological relationships of the forest. At the same time it is evident that much still remains to be done. So many aspects have to be considered and so many factors are involved that it is usually not possible to extrapolate findings from one area to another; in this respect the use of regression analysis to define certain underlying relationships has a very promising future. It is also clear that most countries do not look at the forest simply as a potential drain on water supplies, to be avoided wherever possible. As H. C. Storey, director of the U.S. Forest Service Division of Watershed Management Research, pointed out, the emphasis must be on the multiple use of the forest; Lambor (Poland) echoed the same theme in his recommendations for a water economy plan based on the proper appreciation of the comprehensive role of a forest cover.

Following the symposium, an excursion was made to various forest catchment experiments in the area. Eidmann (Düsseldorf) demonstrated two of his seven stream-gauge installations set up in small catchments to investigate the influence of various types of forest cover and of forest practice, primarily on water yield; at Lahnhof, beech and spruce forest are being compared and at Helgersdorf, coppice and high forest. The Ruhrtalsperrenverein, the organization largely responsible for supplying water to the Ruhr industries, has nine similar installations, two of which, on the Runkhauserbach (99 per cent forest) and the Königswasser (97 per cent arable), were also inspected.

Coming from a country with similar water problems, but without a single catchment experiment designed specifically to investigate forest influences, one is greatly impressed by the enthusiasm with which these problems are being tackled in Germany. It is not difficult to criticize many of these installations; precipitation measurements, stream-gauge design, the possibility of leaks and questionable comparability of catchments, all raise problems which could readily intimidate the ultra-cautious, particularly in view of the costs involved. Nevertheless, with the example set by the Americans, Germans and other nationals, and with access to their knowledge and experience, can we in Britain afford not to set up similar experiments of our own?

L. Leyton

Lysimeters

A lysimeter is an apparatus used for measuring the quantity or quality of water which has percolated through a container which is filled with soil or similar material. It is easy to see that, within such a definition, lysimeters can be used for such a variety of specific purposes that each installation must be considered on its own merits; an ever-present danger is to interpret what is measured by means of the lysimeter as being representative of any conditions other than those obtaining in the lysimeter itself.

Lysimeters are commonly installed to throw light on what happens in the field, where many different factors affect the amount of percolated water. The position is essentially similar to that encountered in measuring temperature, rainfall and other meteorological factors, where, however, arbitrary standards of measurement have been accepted for purposes of making comparisons between values obtained at different sites. So far, standard conditions have not been accepted for lysimeter installations and readings, and indeed comparatively few suggestions have been put forward for standardization of observations.

It is thus not surprising that several of the papers read at the symposium described lysimeters which could throw some light on what happened, with the passage of time, only in particular circumstances. Various ingenious and, in some cases, expensive installations have been set up, in which care has been taken to avoid such things as disturbance of the natural soil profile, or of the homogeneity of the vegetation cover. But in almost none were there lacking unmeasured variables which, even though in some cases a correlation with adjoining field conditions could satisfactorily be established, allowed of any trustworthy comparison between one site and another.

W. C. Visser, of the Netherlands, was one of those who read papers pointing out that conditions in a lysimeter are essentially artificial, and that the factors introduced by this artificiality need to be measured or eliminated. Visser particularly recommended water-flow potential measurements in the field to 'calibrate' the lysimeter measurements; this involves measuring ground-water depth, tension in the capillary zone, tensions in the plants, and vapour pressures in the air. K. Ubell, of Hungary, placed emphasis on the need to have constant records of the temperature gradients in the field and in the lysimeter. Harrold and Dreibelbis, in describing some of the work at the well-known installations at Coshocton, Ohio, showed themselves vividly aware of difficulties, which have failed to be understood by some who have quoted the Coshocton results too uncritically.

Lysimeters can broadly be divided into those measuring volume and those measuring weight. There is much to be said for the latter, because not only do they help to overcome the problem of changes in the amount of water stored in the lysimeter, but they also enable changes to be recorded as continuously as is desired; lysimeters working on volumes of outflow water necessarily have by contrast a much more considerable time-lag. Several speakers, however, pointed out that weighing lysimeters were not the complete answer which some had incautiously considered them to be; for example, one is not sure whether what is being weighed at one season is strictly comparable to that being weighed at another time of the year.

E. J. Winter (Great Britain) read a joint paper by P. J. Salter, G. Stanhill and himself describing the installations at the National Vegetable Research Station in Warwickshire. Besides directing attention to some interesting and significant results which need further investigation, he stressed that much satisfactory and adequate practical advice can now be given to growers, even though more research is needed to elucidate the mechanisms of, and variations in, the water balance.

G. F. Makkink, in describing the various installations in the Netherlands, made the following useful summary remark: "It is considered the final aim of lysimeter research to gain an insight into the water balance of any natural soil-profile as a function of climate, vegetation and movement of the ground water. This aim widely surpasses the limited scope of the lysimeter observation of the separate institutions who own them". The recognition of this has led to appreciable progress in the Netherlands in co-ordinating results.

Several speakers, particularly G. L. Dupriez (Belgian Congo) and F. H. W. Green (Great Britain), emphasized the value of first obtaining observations of potential evapo-transpiration, under 'standard' conditions, at a network of stations. Cheap oil-drum lysimeters were found to be quite adequate for this purpose, if both sited and handled under comparable conditions. Green pointed out that, by simple subtraction, one could get reasonably reliable values of the seasonal differences in 'water deficit' and 'water surplus' from station to station, even where the absolute values of potential evapo-transpiration and of rainfall were open to doubt. In this connexion, several speakers emphasized the difference between the rainfall measured in rain-gauges at the standard height and that falling on the ground. In both Britain and Germany this seemed to be of the order of rather more than 5 per cent; ground-level gauges are therefore installed at most German lysimeter stations.

One of two week-end excursions at the end of the symposium was devoted primarily to visiting lysimeter installations. The first of these visited was in the Senne heathlands, near Bielefeld, and was operated in connexion with the water undertaking of that town. It consisted of four weighing lysimeters, one metre square cross-section, filled (in three cases) with 'monolith' blocks from three different soil profiles found in the area (the fourth was a 'disturbed' block from one of the areas). None of these lysimeters was irrigated, so that, particularly in a dry year like 1959, the lysimeters could supply facts but not explanations.

The second place visited was to an ingenious forest installation at Bossendorf, near Haltern, in Westphalia. Here, in addition to a more orthodox

lysimeter in an adjoining arable field, were two batteries of four lysimeters each, one under a stand of conifers and one under a stand of broad-leaved trees. These had been made by pressing large rain-gauge-shaped lysimeters upwards into the soil under the trees, from a horizontal gallery, painstakingly excavated to avoid disturbance of the natural conditions. The aim was limited to measuring differences in rates of recharge of soil-water, but they have not been operating long enough for the results to be assessed.

The third visit was to the installation of the Dortmund Waterworks at Geisecke on the Ruhr. Here there are batteries of (*a*) volume lysimeters, and (*b*) weighing lysimeters, filled with different materials and with different vegetation covers. Although undoubtedly useful information is obtained, the results from type (*a*) might be queried in view of the very large amount of bare concrete surrounding the tanks, while in both types (*a*) and (*b*) reasonable homogeneity with the surrounding vegetation was achieved only in certain of the tanks. In fact, the series of records from each of these lysimeters, as at some other installations, could be considered only separately, and not safely compared with those from any of the others.

F. W. GREEN

OBITUARIES

Prof. H. J. Backer

HILMAR JOHANNES BACKER was born at Dordrecht on January 13, 1882, and died at Glimmen, near Groningen, on April 29, 1959. He was a pupil at the Gymnasium in Dordrecht and studied at the University of Leyden under Franchimont in chemistry and H. A. Lorentz in physics. His doctoral thesis was entitled "De Nitraminen en hunne Electro-chemische Reductie". He also worked with Elbs at Giessen on the technique of electrochemical reduction and later in the Davy Faraday Laboratories at the Royal Institution in London. After two to three years on the staff at Leyden and a similar period in industry and in Government service, he was called to the chair of organic chemistry at Groningen in 1916 as successor to J. F. Eykman. His colleague, Dr. J. Strating, said that Backer soon showed his capacity for hard work, for concentration and for utilizing every free moment, and yet it seemed that the passage of years only increased his broad humanity.

A survey of his researches (1905–55) reveals an increasing occupation with compounds containing the $-SO_2-$ group. This can be correlated with the early work of Franchimont on α-sulphopropionic acid which Backer and Franchimont resolved in 1914 by means of strychnine. Backer then prepared and resolved α-chloro- and α-bromo-sulphoacetic acids, α-seleninopropionic acid, α-sulphobutyric acid, several α-arsenocarboxylic acids and also chlorobromoacetic acid.

About 1930 he began a study with Strating of the unsaturated cyclic sulphones formed from sulphur dioxide and butadienes. The close relation of these compounds to the thiophens led to a study of the oxidation of thiophen homologues in the hope of obtaining the corresponding sulphones. Thiophen itself, on oxidation, gives a 'sesquioxide', presumably formed by 1 : 4-addition between the unstable sulphoxide and sulphone of thiophen. It was found that certain dialkyl- and diphenyl-thiophens gave sulphones on oxidation; tetraphenylthiophen had long been known to do so. This was attributed to the presence of the substituents which increased the electron availability on the sulphur atom. In some cases a sesquioxide was formed. On the other hand, electron-attracting groups in the benzene nucleus of 3 : 4-diphenylthiophen inhibited sulphone formation.

In 1952 Backer began to study imenes of the type $(R\cdot SO_2)_2C{=}C{=}NR'$, which are very reactive. Three of these have recently been submitted to X-ray analysis by Wheatley, Bullough and Daly in Leeds with very interesting results.

At the beginning of the occupation of the Netherlands in 1940, Backer gave much help and advice to his students, whose whole world had suddenly fallen to pieces. He kept in touch with all those who were forcibly deported to Germany, sent them parcels and encouraged and advised their parents. When I visited Backer in 1947, he referred only briefly to his imprisonment in 1945 and said nothing of the physical violence which he endured during interrogation. The other occupant of his cell was shot. The liberation of Groningen probably saved Backer from the same fate.

Later on, two of my research students visited Groningen at Backer's invitation, lived in his house for some weeks and worked in his Department. Dr. A. Ash wrote: "It was his habit to have students lodging with him and he liked nothing better than for students to call in the evening for a short talk; help or advice were gladly given. He interested himself also in their social life. He methodically inquired of his students' progress every morning and imparted an impressive practical technique particularly rich in devices for facilitating manipulation of small quantities". Dr. A. G. Lowther has said: "One's immediate impression on meeting him was that here was a man who demanded one's respect and affection. This was not only a first impression—it was there among his students—they had a real affection, almost love, for Prof. Backer. He never appeared to be hurried, was most courteous and had a quiet, but real, sense of humour. I never heard one word of criticism of him . . . there was a serenity about him and his house".

Dr. Strating wrote: "Nothing could prevent him from helping a friend or a student who was in need, and this, no doubt, led to his arrest". Backer was a knight of the Order of the Netherlands Lion, a member of the Royal Academy of Sciences in Amsterdam, a correspondent of the Paris Academy of Sciences and an honorary member of the Solvay Institute in Brussels. He received honorary doctorates from the Universities of Ghent and Lille.

When those who knew him recall Backer's achievements as a man and as a chemist, his great capacity for friendship and for hard work, his fearlessness and his quiet mind, they feel that he went far towards solving happily the eternal problem, *si jeunesse savait, si vieillesse pouvait.*

FREDERICK CHALLENGER

Prof. E. S. Salmon

After a long illness, Prof. Ernest Stanley Salmon died on October 12 at the age of eighty-eight.

Anyone unfamiliar with mycology might be pardoned for thinking that his reputation rested on the breeding of new varieties of the hop (*Humulus lupulus* L.). Yet when he came to Wye College (University of London) in 1906, he had already established himself as the authority on an important group of fungi. Researches at the Jodrell Laboratory, Kew, had led to the publication, in 1900, of "A Monograph of the Erysiphaceae", a work which remains fundamental for systematic mycology and which was reproduced as microcards only a few months ago. After further study in the laboratory of Prof. Marshall Ward at Cambridge, he demonstrated the very highly developed specialization of parasitism in the same group; accounts of this may be found in the *Transactions of the Royal Society*, 1904 and 1905.

Salmon was appointed to Wye College to study diseases of plants and so was probably the first professional plant pathologist in Britain. There were then few helpful text-books, but it was not long before he had combined laboratory work and field experiments to good purpose. His first papers, which may even now be read with profit, describe in some detail a selection of the diseases of crop plants; the main emphasis being on apple scab (*Venturia inaequalis* (Cooke) Wint.) and other pathogens of fruit. Bordeaux mixture and lime sulphur, fungicides which are still in use, were introduced to Kentish orchards, while he conducted a vigorous campaign in the Press and elsewhere which led to the passing of the Destructive Insects and Pests Act in 1907.

He was elected president of the British Mycological Society in 1911; appointed reader in economic mycology in the University of London in 1912, professor of mycology in 1925 and emeritus professor in 1939. He was made Fellow of Wye College in 1948.

When, soon after the First World War, the specialist advisory service in Britain began, he was appointed advisory mycologist of the Wye Province. Until he relinquished this appointment in 1937, he led a series of investigations on diverse plant diseases. Particularly noteworthy are the contributions on fruit and hops with which are associated his colleagues, Drs. H. Wormald and W. M. Ware.

Salmon arranged a programme of hop breeding in 1907 and followed this without a break until his last illness. From the beginning, one of his main aims was to produce varieties with exceptionally high preservative values; such varieties would enable the British grower to compete with imported American hops. In order to have at command a wide range of diverse types, he assembled male and female plants from different parts of the world; this unique collection, together with selected progeny, for long occupied about an acre of ground at Wye College. Of the many thousands of seedlings raised, his earliest success was the English-grown American, Brewer's Gold. This was *F*1 from an English male hop and a wild hop from Morden, Manitoba. Paradoxically, the variety has been more widely grown in Canada and the United States than in Britain. The varieties Bullion and Northern Brewer which have become so popular with the British hop industry during the past decade are *F*1 and *F*3 seedlings, respectively, of parents from the same sources. Other seedlings of his raising proved to be tolerant to hop wilt (*Verticillium albo-atrum* Reinke and Berth.) and these have been of great value for planting in infected soil.

In recognition of his work on hops he received in 1955 the Horace Brown Medal, the highest honour which the Institute of Brewing can bestow.

After the manner of the pioneer, Salmon was a strong individualist and, in his later years at least, showed little enthusiasm for gatherings, whether scientific or social. He found relaxation in literature and in his small garden of rare plants.

H. H. Glasscock

Prof. J. Zenneck

Prof. Jonathan Zenneck, one of the earliest pioneers of radio science, died in Munich in April, a few days before his eighty-eighth birthday. Prof. Zenneck was born in 1871 in Württemberg, Germany, and studied mathematics and natural science at Tübingen, where he obtained his doctorate in 1894. In the following year he became an assistant in the Physical Institute in Strasburg; he moved to Dantzig in 1905 to become assistant professor, and later (1911) professor of physics in the Institute of Technology. His academic career was continued by his appointment in 1913 to the chair of physics at the Technical High School of Munich, where he remained until his retirement.

Most of the basic contributions of Prof. Zenneck were made in the days when spark transmitters were used for wireless telegraphy, concurrently with the development of high-frequency machines and arc generators to produce undamped oscillations for radio-telephony. Among the earliest of his achievements was the establishment of the first radio-link for navigational purposes between Cuxhaven and Heligoland in 1899–1900. His basic experimental and theoretical contributions to wave propagation were of great importance in the early development of wireless communications. He expounded the first theory on wave propagation along the Earth (Zenneck wave) which explained the effect of the ground constants on polarization and absorption of the waves. Also well known are his basic contributions to ionospheric research, which he initiated in Germany; and he was the founder of the first German ionospheric research station, Herzogstand, in Kochel, Bavaria, which was in operation until 1945.

Dr. Zenneck's interests were not limited to radio. He also contributed to other areas in the general field of applied physics such as acoustics and gas discharges. He was the author of the first German text-book on wireless telegraphy, "Electromagnetic Oscillations and Wireless Telegraphy", published in 1906, which was the classic work in this field for many years. He was also the editor of the *Hochfrequenztechnik und Elektroakustik*, the leading journal of the world during the early days of radio.

His second book, entitled "Wireless Telegraphy", was published in Germany in 1908, and a second edition appeared four years later. It was translated into English in 1915 by A. E. Seelig, and published in London and New York, forming one of the most useful text-books available to students at that time on the generation, propagation and detection of electromagnetic waves in the radio part of the spectrum.

He received many honours and medals from academic and professional societies, including an honorary doctor's degree from the Institute of Tech-

nology at Dresden. He was made a fellow of the Institute of Radio Engineers (N.Y.) in 1915, received its medal of honour in 1928, and was a member of the board of directors and vice-president in 1933. He was honorary president of the German National Committee of the International Scientific Radio Union, and was elected vice-president of that Committee in 1938. All those who had the pleasure of meeting Prof. Zenneck at international conferences will remember his charming personality and his modest simplicity, combined with wittiness and quickness of repartee. R. L. SMITH-ROSE

NEWS and VIEWS

Nobel Prize for Physics for 1959: Dr. Emilio Segre and Dr. Owen Chamberlain

Dr. Segre and Dr. Chamberlain, who have been awarded the Nobel Prize for Physics for 1959, have collaborated in research in high-energy physics for a number of years at the Lawrence Radiation Laboratory of the University of California at Berkeley. Dr. Segre was born in Italy in 1905 and was a member of Fermi's remarkable nuclear research school in Rome. He emigrated to the United States before the War. Dr. Chamberlain is thirty-nine and, like Dr. Segre, is a distinguished experimentalist. Their collaboration at Berkeley has been associated with the great accelerators which have been built there over the years. They were the leading members of a team engaged in experiments on nucleon-nuclear interaction with the help of the 184-in. synchrocyclotron, and in particular made a detailed study of polarization phenomena in high-energy scattering. The work which has now been honoured by the Nobel award has, however, been their discovery in 1955 of the antiproton in experiments with the 6 GeV.-proton synchrotron, the bevatron. The existence of the antiproton had been confidently postulated for many years; the discovery of the positron in 1932, and its interpretation on the Dirac theory, also implied the existence of other anti-particles including a negatively charged proton. Antiprotons stubbornly eluded discovery in the very high-energy (but very low-intensity) bombardments of cosmic radiation, and one reason for building the bevatron was to have an intense beam of sufficiently energetic protons to create proton–antiproton pairs in the laboratory.

The actual discovery, however, involved a long series of painstaking experiments with very elaborate detection equipment which had to be specially developed. The development of effective techniques for beam separation and detection for use with machines of very great energy is a field of research in itself. The antiprotons produced when a beam of high-energy protons falls upon a target in a machine like the bevatron are very greatly outnumbered by other charged particles produced with very broad momentum spectra in the relativistic region. These background particles would cause impossible confusion in the detection apparatus if they were not systematically eliminated. Their elimination in the experiments of Segre and Chamberlain was a major programme in experimental physics. The final apparatus used was elaborate and refined. Charge and momentum separation of antiprotons from the mixed beam of particles was achieved by magnetic deflexion and focusing in separate lenses, and velocity selection was aided by time-of-flight and Čerenkov radiation techniques. When some forty events had been accumulated which corresponded within acceptable margins of error with the properties of the antiproton, it could be said that the antiproton had been discovered. As is usual in high-energy research with large machines, team-work by large numbers of physicists and engineers was involved; the award of the Nobel prize to Segre and Chamberlain underlines the fact that the brilliant individual worker is still needed to inspire and direct the work.

Royal Society Medals for 1959

THE following awards of medals have been made by the President and the Council of the Royal Society: *Copley Medal* to Sir Macfarlane Burnet, director of the Walter and Eliza Hall Institute, Melbourne, Australia, for his distinguished contributions to knowledge of viruses and of immunology. *Davy Medal* to Prof. R. B. Woodward, of the Department of Chemistry, Harvard University, Cambridge, Massachusetts, for his distinguished researches in organic chemistry and particularly for his contributions to the structure and synthesis of natural products. *Hughes Medal* to Dr. A. B. Pippard, reader in physics in the University of Cambridge, for his distinguished contributions in the field of low-temperature physics.

Genetics at Cambridge: Prof. J. M. Thoday

A GRADUATE of the University College of North Wales, Bangor, Dr. J. M. Thoday, who has recently been appointed to the Arthur Balfour chair of genetics in Cambridge, began his research career in the Botany School, Cambridge, under the guidance of Dr. (now Prof.) D. G. Catcheside. These early studies of the action of ionizing radiations on chromosome structure were interrupted by war service in the Royal Air Force, to be resumed after the War at the Mount Vernon Hospital and Radium Institute, where, in collaboration with Dr. J. Read, Thoday demonstrated the effect of oxygen tension on the frequencies of chromosome changes following irradiation. During 1947 he moved to Sheffield to take up an appointment as lecturer in cytogenetics, becoming senior lecturer in charge of the newly founded Department of Genetics there in 1954. In Sheffield his research moved towards the field of population genetics, particularly in relation to the genetical control of stability in development and to the action of disruptive selection. His experiments with *Drosophila* have been especially rewarding in the light they have thrown on the power of disruptive selection to conserve variability and to build up polymorphisms within populations. They are showing us for the first time under controlled conditions how polymorphisms can come into being and how their genetical structure reflects the selection which has brought them about. Prof. Thoday's breadth of experience and originality of approach augur well for the future of genetics in Cambridge.

Genetics at Sheffield: Dr. J. A. Roper

DR. J. A. ROPER returns to his own University (Sheffield) as the first holder of the new chair of

genetics. Dr. Roper graduated in chemistry at Sheffield in 1945, and was trained in bacterial biochemistry under Krebs and McIlwain. He joined the Genetics Department at Glasgow in 1948 and played a very important part in the development of that school of genetics. For his ability as a teacher, and his engaging personality, he is well liked by the students. At Glasgow he introduced, with great success, group discussion methods. Dr. Roper was Rockefeller Fellow in 1953 at the California Institute of Technology, and has lectured at various times in a number of American and European universities. He is secretary of the Genetical Society.

Dr. Roper's training in chemistry and microbial biochemistry was a good foundation for the research in microbial genetics on which he embarked at Glasgow. He played a major part there in a team working on the genetics of *Aspergillus nidulans*. A most decisive contribution was the first deliberate search for, and his demonstration of, what could be called the 'splitting of the gene' (*Nature*, **166**, 956; 1950). Soon after he designed a way of synthesizing heterozygous diploid strains in filamentous fungi. This was the first step which made possible the discovery of the 'parasexual' cycle and the development of genetic analysis *via* mitotic segregation. In the past three years Dr. Roper has become interested in investigating extra-nuclear inheritance in *Aspergillus*. Dr. Roper's ability in teaching and research will be given full opportunities in this new chair, born in a most favourable environment.

The Animal Health Trust

H.M. the Queen has graciously consented to become patron of the Animal Health Trust. The Trust was founded by Dr. W. R. Wooldridge in 1942 for the purpose of improving the general health standards of all types of domesticated animals, and it approaches the task in two ways. First, by means of scientific investigation of the many disease problems of such animals; secondly, by stimulating the flow of trained personnel into the ranks of the veterinary profession through financial grants to needy students and by furthering the higher education and specialization of veterinary graduates. Four research centres have been established by the Trust for the study of disease in horses, dogs, poultry and farm animals, respectively, the latest of which—the Farm Livestock Research Centre at Stock, Essex—was opened by H.R.H. Prince Philip in December 1957 (see *Nature*, 181, 76; 1958). A new surgical unit to mark the patronage of the Queen is to be built at the Equine Research Station, Newmarket, at a cost of about £30,000.

The Office of the Lord Privy Seal

With the appointment of Lord Hailsham as the Minister with general responsibility for science and technology, including atomic energy, the Atomic Energy Office and the Lord President's Office will be combined. The new Office will be in the charge of Mr. F. F. Turnbull, whose appointment as deputy secretary, to succeed Sir Friston How in charge of the Atomic Energy Office, was announced some months ago. It will be organized in two Divisions: (1) a General Division, under Mr. R. N. Quirk, under-secretary, corresponding to the previous Lord President's Office; and (2) an Atomic Energy Division, under Mr. M. I. Michaels, under-secretary, corresponding to the previous Atomic Energy Office.

Parliamentary Responsibility for Science and Technology

The Prime Minister made a statement in the House of Commons on October 30 regarding the responsibilities of the Lord Privy Seal and the Minister for Science, stating that other Ministers would remain responsible for the scientific establishments within their own Departments, but that the Minister for Science was to be responsible for broad questions of scientific policy outside the sphere of defence, and was advised by the Advisory Council on Scientific Policy on general questions which relate to the whole field of civil science. On November 3, Mr. Macmillan indicated that the arrangements for answering questions in the House of Commons which fall within the responsibility of the Minister for Science would be as follows: questions about the Medical Research Council and radiobiological hazards, the Minister of Health; Agricultural Research Council and Nature Conservancy, the Minister of Agriculture; nuclear energy, the Department of Scientific and Industrial Research and general scientific matters, the Minister of Education; space research, the Minister of Aviation. Where questions about the development of nuclear energy relate to matters for which some other Minister is responsible, that Minister would answer them. For example, questions about nuclear-powered merchant shipping would normally be answered by the Minister of Transport, and about nuclear power stations by the Minister of Power. Mr. Macmillan had earlier defended the exclusion of the new Minister of Power from the Cabinet, but neither that arrangement nor those for answering questions for the Minister of Science in the House of Commons were well received.

Scientific Developments in Britain

Replying to a question in the House of Commons on November 2, Mr. J. B. Godber, as representing the Lord Privy Seal, said that a small temporary station is to be established near Cambridge to expand the work already being done at the Low Temperature Research Station and elsewhere, and he hoped it would be in operation by the end of next year. Meanwhile, the Agricultural Research Council is considering the wider issues raised by the proposal to establish a permanent centre for meat research. In a written answer on November 5, the Minister of Education, Sir David Eccles, as representing the Minister for Science, stated that the new Hydrodynamics Laboratory of the National Physical Laboratory had cost about £2 million, and its staff was nearly 70, including 10 scientific officers. The Laboratory would provide up-to-date facilities for testing ship designs, particularly in reproducing more realistic sea conditions, and research would be conducted to extend basic knowledge of the resistance, propulsion and sea-going qualities of ships, while new ship designs would be tested for industry.

In a written reply to a further question on November 5, Sir David Eccles said that commissioning trials of the fast reactor at Dounreay had proved more difficult than expected, due to chemical engineering problems, but enriched uranium is now being loaded and the reactor is expected to become critical within the next few weeks. This reactor, he said, is an experiment in the development of fast breeder systems, and successful exploitation will depend upon the operating results: the development of the fast reactor will occupy the resources of the Dounreay

establishment for many years. The Admiralty submarine reactor development programme at Dounreay is now getting under way, and firms in the north of Scotland with the necessary scientific and technical experience would be given the opportunity of tendering for any development work which could be let out to industry.

Inauguration of *Merlin*

WHEN H.R.H. the Duke of Edinburgh formally inaugurated *Merlin*, the nuclear research reactor built by Associated Electrical Industries Ltd. (see *Nature*, September 5, p. 11) at Aldermaston on November 6, an underwater closed-circuit television camera specially designed by E.M.I. Electronics Ltd. made possible the observation of activity inside the reactor. This camera, which is 30 in. long, $3\frac{1}{2}$ in. in diameter, and enclosed in a pure aluminium water-tight housing, is a permanent part of the equipment of *Merlin*. In addition, a standard E.M.I. camera has been mounted above the reactor, on the second floor of the reactor hall. This provides a continuous view all around *Merlin*, and ensures that no unauthorized staff are near the reactor when it is in use. Two 14-in. receivers, one for each camera, have been rack-mounted in the control room on the ground floor and another receiver has been installed on the second floor for observation inside the reactor. A 17-in. console receiver, capable of selecting a picture from either camera, has been installed in the reactor conference room. Three more E.M.I. cameras and seven more receivers were used at the opening ceremony to give the audience an uninterrupted view of the proceedings from the moment the Duke of Edinburgh entered the reactor hall on the ground floor, while he was inspecting the top of *Merlin* from the second floor, and upon his return to the ground floor to start the reactor and unveil a commemorative plaque.

Joint British Committee for Vacuum Science and Technology

FOLLOWING the Institute of Physics' London Conference on high vacua held in April last, various suggestions were made for arranging regular meetings in Britain on vacuum science and technology, and for British participation in international conferences in this field. As a result of informal discussions, a Joint British Committee for Vacuum Science and Technology has now been formed. The Committee consists of representatives from each of the following bodies: Institute of Biology, Institution of Chemical Engineers, Royal Institute of Chemistry, Institution of Electrical Engineers, Iron and Steel Institute, Institution of Mechanical Engineers, Institute of Metals, Institute of Petroleum, Physical Society and Institute of Physics. Its objects are: (*a*) to co-ordinate and help to initiate meetings in the whole field of vacuum science and technology arranged by constituent bodies; and (*b*) to act in the collective interest of the constituent bodies by maintaining liaison with the International Organization for Vacuum Science and Technology and with national vacuum societies, and otherwise. The Institute of Physics has agreed to provide the secretariat for the joint committee; communications should be addressed to the Secretary of the Joint British Committee at 47 Belgrave Square, London, S.W.1.

Scientific Policy in South Africa

THE first and second annual reports of the Advisory Council on Scientific Policy of the Union of South Africa cover the periods December 1956–December 31, 1957 (pp. 5) and January 1–December 31, 1958 (pp. 7), respectively (Pretoria: Advisory Council on Scientific Policy, 1958 and 1959). The Council, which has nine members, including Prof. H. O. Mönnig as chairman, was appointed late in 1956 to enquire into and report on all matters referred to the Council by the Minister of Economic Affairs. It is required to acquaint itself with scientific developments and policy in other countries and to advise the Minister concerning any action necessary in South Africa; to take note of all international scientific conferences and make recommendations regarding representatives; and to investigate cases of overlapping of research and other activities referred to the Council by the Minister and make recommendations as to responsibility for the work.

In its first report the Advisory Council, attributing the shortage of scientists and technologists fundamentally to weaknesses in secondary education, recommended that the Government should increase both funds and facilities available for training scientists at the universities as well as actively promote the training of technologists and improve the salaries and conditions of employment of scientists. A survey of the country's requirements for scientists and technologists by the Research Bureau of the Department of Education, Arts and Science was also recommended, and the Council's consideration of the condition and organization of research issued in a recommendation for the appointment by the Government of a commission to investigate the organization of agricultural research and the establishment of an independent Agricultural Research Council. The Advisory Council does not consider its responsibility regarding attendance at international conferences can be satisfactorily discharged under the present arrangements or that the set-up permits adequate access in general to Cabinet level.

In its second report the Advisory Council recommends the compilation and maintenance by the National Bureau for Educational Research of a National Register of Scientists and Technologists. Some consideration was given to the relation between the Council for Scientific and Industrial Research and the universities, and at the request of the South African Chemical Manufacturers' Union the Council considered the desirability of Government support for research on the processing of agricultural products, but concluded that the proposal was undesirable and that developments in this field should be left with the Council for Scientific and Industrial Research. An inquiry into overlapping of State-aided research, requested by the Treasury, led to the recommendation that the Government Metallurgical Laboratory should become an institute of the Council for Scientific and Industrial Research. A Cabinet Committee has been appointed to investigate the shortage of man-power in science and means of reducing this shortage, and a commission is investigating the desirability of an independent Agricultural Research Council, but otherwise no action appears to have been taken on the recommendations suggested in the first report.

Inland Telegraph Service

THE Committee appointed during December 1957, under the chairmanship of Sir Leonard Sinclair, "to advise the Postmaster-General on the future place of the inland public telegraph service as part of the

communication facilities of the United Kingdom", does not consider that, subject to keeping under review the extended use of the telephone for sending telegrams over the shorter distances, further integration of telegraphs with telephones, as recommended by the Bridgeman Committee of 1932, would be justified at present, though there may be scope for further integration with the telephone service or possibly with the telex service later. The number of telegrams has steadily declined from 53·3 millions in 1947–48 to 18·7 millions in 1956–57 and an estimated 16·9 millions in 1957–58, and the loss per telegram has risen from 14·2*d.* to 40·3*d.* in 1956–57 and 48·2*d.* in 1957–58. This is mainly due to the relatively small gap in the communications facilities of the United Kingdom filled by the service and this gap is being reduced in size by other Post Office services. Foreign administrators face a similar problem and the traffic is expected to continue to decline. Although the service is only of marginal importance to business establishments and only occasionally used by the public for social purposes, in its report the Committee recommends retention of the service to handle a proportion of overseas and emergency telegrams and for other reasons (Report of the Advisory Committee on the Inland Telegraph Service, 1958. Pp. iii+11. London: H.M. Stationery Office, 1958. 1*s.* net). Increase of the tariff is recommended and the new structure should be one of a basic charge plus a charge for every word. The Post Office, it is suggested, should consider the elimination or reduction of the deficits due to Press traffic and telegrams to the Irish Republic and should arrange to charge the British Transport Commission with the cost of Railway Pass telegrams.

Meldola Award

It is announced that the next award of the Meldola Medal will be made early in 1960 to the chemist who, being a British subject and under thirty years of age at December 31, 1959, shows the most promise as indicated by his or her published chemical work brought to the notice of the Council of the Royal Institute of Chemistry before December 31. The merits of the work may be brought to the notice of the Council, either by persons who desire to recommend the candidate or by the candidate himself, by letter addressed to the President, Royal Institute of Chemistry, 30 Russell Square, London, W.C.1, from whom further information can be obtained.

Royal Society of Edinburgh: Officers for 1959-60

At the annual statutory meeting of the Royal Society of Edinburgh the following officers and members of Council were elected: *President:* Prof. E. L. Hirst, Department of Chemistry, University of Edinburgh. *Vice-Presidents:* Dr. D. P. Cuthbertson, Mr. A. W. Young, Prof. T. Neville George, Prof. J. R. Matthews, Dr. T. R. Bolam, Dr. Douglas Guthrie. *General Secretary:* Prof. Norman Feather, Department of Natural Philosophy, University of Edinburgh. *Secretaries to Ordinary Meetings:* Dr. A. W. Greenwood and Dr. Mowbray Ritchie. *Treasurer:* Dr. J. R. Peddie. *Curator:* Dr. R. Schlapp. *Councillors:* Prof. A. M. MacBeath, Prof. R. A. Rankin, Prof. A. E. Ritchie, Prof. V. C. Wynne-Edwards, Prof. E. G. Cullwick, Prof. G. Pontecorvo, Prof. M. M. Swann, Prof. H. A. Brück, Prof. T. S. Westoll, Dr. H. R. Fletcher, Prof. G. L. Montgomery, Prof. W. L. Weipers.

Announcements

The Minister for Transport and Power for the Republic of Ireland has appointed Mr. J. Connor, of the Department of Transport and Power, to be chairman of the National Committee for Geodesy and Geophysics in succession to Mr. A. Ó. Coinneain.

Mr. R. Levin has been appointed development planning executive at Aspro-Nicholas, Ltd., of Slough, Bucks. Mr. Levin was formerly chief pharmacist, Research and Development Division, and lately manager of the Technical Information Department of the Distillers Co. (Biochemicals), Ltd. He is the author of "The Pharmacy of Silicones and their Uses in Medicine".

Following the resignation of Dr. C. E. Dalgliesh as secretary of the Biochemical Society, Dr. P. N. Campbell, Courtauld Institute of Biochemistry, Middlesex Hospital, London, becomes secretary to the Committee, and Dr. W. J. Whelan, Lister Institute, Chelsea Bridge Road, London, S.W.1, has been appointed meetings secretary.

The National Collection of Industrial Bacteria has been transferred from the National Chemical Laboratory to the Torry Research Station (Department of Scientific and Industrial Research). All future inquiries and correspondence concerning the Collection should be addressed to the Curator, National Collection of Industrial Bacteria, Torry Research Station, Aberdeen.

A symposium on haematology has been organized by the University of Cambridge Post-Graduate Medical School, and will be held during December 7–9. Further information can be obtained from the Secretary, Medical School, Tennis Court Road, Cambridge.

The U.S. Office of Naval Research has announced that the second Conference on Semiconductor Surfaces will be held at the U.S. Naval Ordnance Laboratory, White Oak, during December 3–4. Further information can be obtained from the chairman of the Steering Committee, Dr. J. N. Zemel, U.S. Naval Ordnance Laboratory, White Oak, Silver Spring, Md.

The Indian Society of Theoretical and Applied Mechanics has announced the Fifth Congress on Theoretical and Applied Mechanics, to be held at the University of Roorkee during December 23–26. Further information can be obtained from the Secretary-Treasurer, Indian Society of Theoretical and Applied Mechanics, Institute of Technology, Kharagpur, India.

The autumn general meeting of the British Iron and Steel Institute will be held in London during December 2–3. The sessions, some of which will run simultaneously, will be held in the Great Hall, Caxton Hall, S.W.1, and in the Hoare Memorial Hall, Church House, S.W.1. Further information can be obtained from Mr. K. Headlam-Morley, Iron and Steel Institute, 4 Grosvenor Gardens, London, S.W.1. All applications must be returned by November 27.

Erratum. In the article entitled "Transmission of a Virus to Strawberry Plants by a Nematode (*Xiphinema* sp.)" in *Nature* of September 26, p. 962, line 5 in par. 2 should not have been inserted. The phrase should read: ". . . the causal virus of yellow crinkle and of mosaic was transmitted by mechanical inoculation from plants of a number of strawberry varieties, including . . .".

INTERNATIONAL UNION OF PURE AND APPLIED CHEMISTRY

COMMISSION ON GEOCHEMISTRY

THE Commission on Geochemistry of the International Union of Pure and Applied Chemistry held a meeting in Munich during August 26–27, during the twentieth Conference of the Union. Attending the meeting were the following members of the Commission: Prof. T. F. W. Barth (*president*), Dr. F. M. Vokes (*acting secretary*), Prof. C. Burri, Prof. C. W. Correns, Prof. S. I. Tomkieff, Prof. L. R. Wager, Prof. F. E. Wickman and three observers of the Commission: Prof. A. P. Vinogradov, Prof. K. Sugawara and Prof. E. Ingerson.

The Commission discussed at length its future objects and aims, particularly with regard to the work of its sub-committees. The chairmen of the three existing sub-committees reported on their activities; these comprise the subcommittee on abstracting, translation and information; the sub-committee on the chemistry of the oceans and the sub-committee on rock analyses. It was decided to broaden the scope and work of the Commission by forming sub-committees on "the organic compounds in the crust of the Earth" and "the beginning of the biosphere".

It was also considered highly desirable that some form of code should be compiled which could be used to indicate the exact analytical methods which had been employed in mineral and rock analyses reported in publications. It was decided to seek the co-operation of the Sections of Inorganic and Analytical Chemistry of the Union to further this end. The question of education and training for geochemistry was also discussed at some length. In particular, it was agreed that the education committee of the Geochemistry Society should be encouraged to produce a definitive report on this subject as a basis for further discussion.

The subject of future symposia on geochemistry came in for a considerable amount of discussion. It was decided to offer the Commission's co-operation in respect of the proposed symposium of the International Union of Geodesy and Geophysics to be held in Helsinki in July 1960, and that of the Geochemical Society to be held in Copenhagen in August 1960. For the Copenhagen symposium it was suggested that the Commission should be responsible for organizing a section of the geochemistry of sedimentary carbonate rocks.

Prof. A. P. Vinogradov of the Vernadsky Institute of Moscow gave the members present a short summary of the current geochemical work being carried out at the Institute. He also mentioned that discussions were taking place regarding the formation of a Russian geochemical society which he hoped would in time be able fully to co-operate with similar bodies outside Russia. Prof. K. Sugawara also gave a short account of the position and activities of the Japanese Geochemical Society.

Election of Members. In order to replace those members due to retire at the end of the present year, the following were elected members of the Commission: Prof. L. H. Ahrens, Prof. E. Ingerson, Prof. K. Sugawara and Prof. A. P. Vinogradov. The officers elected for the session beginning 1960 were: *President*, Prof. C. W. Correns (Göttingen); *Vice-President*, Prof. A. P. Vinogradov (Moscow); and *Secretary*, Prof. E. Ingerson (Austin, Texas). In addition, it was decided to invite seven new observers to serve with the Commission. The president-elect, Prof. Correns, proposed a vote of thanks to the retiring president, Prof. Barth, for his work for the Commission during his term of office and this was carried with acclamation.

SCIENCE AND PHILOSOPHY

THE fourth annual conference of the British Society for the Philosophy of Science was held during September 25–27 at Newnham College, Cambridge, and was attended by about eighty members and guests. Dr. M. B. Hesse was conference secretary.

Four symposia were held: "Scientific Research and the Philosophy of Science", "Biology and Physics", "Classification, Concept-formation and Language", and "Knowing and Being".

At the first session, with Dr. J. O. Wisdom in the chair, it was argued whether 'philosophy of science' can be held to refer to any activity not properly subsumed under 'scientific research', and, if it can, whether that activity is relevant to research. Prof. H. Dingle mentioned various questions of value and purpose, as well as of method, papers on which were unlikely to be accepted for publication in journals concerned with particular sciences. Prof. H. C. Longuet-Higgins, on the other hand, argued that, of the product of philosophers of science, part was science, part was philosophy, and the remainder was of no use to man or scientist. This aroused some consternation, as members consulted their own credentials and invoked those of others, but in due course a consensus emerged that scientists have to think critically about their thinking, and the comparative study of modes of scientific thought may help them to do so.

In the second session, Prof. J. H. Woodger described an abstractive hierarchy of terms characterized by a one–many relation, and its use as a conceptual framework in biology. With some hierarchies of cells, every cell is a distinct life; with others, only the first cell in each hierarchy, elaboration occurring on subsequent levels. Morphology was the study of the arrangement and differentiation of parts; physiology was the study of the existential dependence of parts. Genetics was concerned with the process in which the first member of a hierarchy results from the conjunction of two members of other hierarchies. Dr. E. M. Hutten set alongside this framework the sequence exhibited by radioactive

decay : each new atom produced was similarly time-extended, stemmed from a parent atom, and consisted of existentially dependent parts, but it was not dependent on its environment analogously to a cell. In elementary-particle theory, explanation might amount to little more than classification, or an enumeration of possibilities, and might have little more predictive power than comparable biological models with their over-determination and multiple-causation. The chairman, Prof. C. F. A. Pantin, referred to the occurrence, in biology, of morphological models that can be interpreted at more than one level in an organizational hierarchy. In discussion it was suggested that the predictive power of a theory might not always be manifest at the time of its original formulation ; it might have to await development of deduction. There was much interest in the relative importance of the past history of an entity in biology and in physics : historical existential dependence appears to be a function of complexity.

In the third session, with Mr. G. Buchdahl in the chair, Miss M. Masterman and Mr. R. M. Needham presented the strategy, and some of the tactics, of a method of analysing language by assimilating it to a library classification system in which concepts are arranged on a finite lattice ordered by a single, weak, 'concordance' (inclusion) relation. They further suggested that the formation of scientific concepts is a development of language according to this model. There was some discussion as to whether the method is a technology for mechanical translation, or a science, or a philosophy of language, and it was suggested that it could be viewed as a scientific model of language, containing the partly uninterpreted concept 'inclusion', and capable of being tested by experiments on translation and on analogy-finding.

In the fourth session, under the chairmanship of of Dr. W. H. Thorpe, Prof. M. Polanyi presented a way of talking about the primary process of knowing by perception, the pre-articulate act of knowing, which partakes of the uniqueness of the individual percipient, the unspecifiable personal knowledge from which any specifiable, potentially public, knowledge is derived by a process of abstraction. Complex entities were commonly perceived and recognized as wholes before particulars had been identified ; the process of discovery, in fact, might be regarded as an alternation of analysis, recognizing particulars, and integration, recognizing the relations of parts to the whole. Prof. R. B. Braithwaite suggested that too narrow a view might be taken of specifiability, and that some levels of subjective experience, however vague, could be conveyed by language—for example, 'Oblomov' conveys the experience of laziness ; beyond that, he differed from Prof. Polanyi in his use of the term 'knowledge' for what was unspecifiable. In the subsequent discussion there was some reluctance to focus on this rather undemocratic mode of tacit awareness, and a preference for talking about what can be made public, with the implication that the progressive refinement of scientific language tends to eliminate the unspecifiable.

In conclusion, it is perhaps worth directing attention to the unusualness of a scientific conference at which speakers are not armed with specified and verifiable data but attend primarily to make as explicit as possible how they think, and to receive criticism of the process thereby revealed, especially from those who are not working in the same field. The coherence of this universe of discourse was illustrated by the frequency of reference from one discussion to another, the *esprit d'escalier* from one session often finding its outlet in a later one ; its range, by the frequency of spontaneous quotation, not only from Shakespeare and Wordsworth but also from Swinburne (and early Swinburne, at that).

G. E. Denyer

FOURTEENTH ANNUAL CALORIMETRY CONFERENCE

THE fourteenth annual Calorimetry Conference, held at Yale University in the Sterling Chemistry Laboratory during September 10–12, was attended by more than one hundred scientists from the United States, Canada and Europe. Under the chairmanship of Dr. David White (Ohio State University) thirty papers were read and discussed. These included : heat capacity measurements at temperatures as low as 0·1° K. and as high as 1,400° K. ; precision reaction and bomb calorimetry ; solution calorimetry ; and determinations of stored energy in solids.

Most of the papers were concerned with recent developments in calorimetry. However, as calorimetric techniques are extended to more extreme conditions, the problems that led to the founding of the Conference remain under new guises. The need for better temperature measuring devices was emphasized in seven papers that reported on research at temperatures below 11° K. No device comparable to the platinum resistance thermometer, now in general use for measurements above 11° K., is yet available for the very low temperatures at which some of the most important calorimetric research is now being done. However, the reports on a device that may extend precision thermometry to at least 1° K., namely, the germanium resistance thermometer developed in the Bell Telephone Laboratories, were received enthusiastically. Twelve of these thermometers had been provided for a calorimetry conference test programme involving eleven different laboratories. Three papers at the Yale conference described the first results of the investigations, which were so promising that the Conference plans to seek a manufacturer of additional units for a more extensive testing programme.

Special addresses were given by Profs. George S. Parks (Stanford University) and Lars Onsager (Yale University). At the annual banquet, Parks delivered the Hugh M. Huffman Memorial Lecture, "Some Remarks on the Thermodynamic Properties of Organic Compounds". Parks and one of his first graduate students, the late Dr. Huffman, started the first systematic calorimetric studies of organic compounds at Stanford more than thirty years ago. Enlivening his remarks with many personal anecdotes, Parks traced the history of thermodynamic research on organic substances and the role improvement

of calorimetric methods has taken in the remarkable progress made in the past three decades. Prof. Onsager gave the principal lecture of the technical sessions on "Co-operative Phenomena", a field in which he has developed much of the basic theory. Many papers at each Calorimetry Conference describe experimental studies of co-operative phenomena, and Onsager outlined the approaches one may take in seeking a theoretical understanding of such effects. Admitting that three-dimensional treatments of critical phenomena by statistical mechanics seem hopelessly complex, he dwelt mostly on more simplified treatments that give results.

In addition to the objective of promoting better calorimetric research, the Conference also is concerned with publication policies relating to calorimetric and thermodynamic articles. A "Resolution regarding Published Calorimetric Data" adopted by the eighth Conference in 1953 has proved to be valuable to editors and authors alike in establishing consistent policies based on the opinions of experts in the field. Because calorimetric research has expanded into many areas not covered by the 1953 resolution, the fourteenth Conference established a committee headed by J. P. McCullough to consider revising and extending the earlier recommendations. Drs. Edgar F. Westrum, jun. (University of Michigan), and Stig Sunner (University of Lund, Sweden) presented a proposal of the Commission on Thermodynamics of the International Union of Pure and Applied Chemistry for a joint meeting in 1961 of the Calorimetry Conference and the Subcommissions on Experimental Thermochemistry and Experimental Thermodynamics. The Conference unanimously approved the proposal for a joint meeting to be held either before or after the biennial meeting of the Union that year in Montreal, Canada. Plans will begin immediately for what should be one of the most important conferences ever held in the field of calorimetry.

At the annual election the following members were appointed to Conference offices: *Chairman*, Dr. J. P. McCullough (Petroleum Thermodynamics Laboratory, Bureau of Mines); *Chairman-Elect*, Dr. D. W. Osborne (Argonne National Laboratory); *Directors*, 1959–62, Dr. N. E. Phillips (University of California, Berkeley) and Dr. J. M. Sturtevant (Yale University). Other officers include: *Secretary-Treasurer*, Dr. C. E. Messer (Tufts University) and *Directors*, Dr. David White, Dr. D. H. Andrews (Johns Hopkins University), Dr. J. E. Kunzler (Bell Telephone Laboratories), and Dr. J. A. Morrison (National Research Council, Ottawa).

SECOND AUSTRALIAN SPECTROSCOPY CONFERENCE

THE second Australian Spectroscopy Conference, convened by Dr. A. L. G. Rees (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) and held in the Chemistry Department of the University of Melbourne during June 1–3, was opened by Prof. J. S. Anderson, who welcomed the 110 participants and the four exhibitors of commercial spectroscopic equipment. The first session of the conference was devoted to ultra-violet spectra and began with a review by Prof. N. S. Bayliss (Chemistry Department, University of Western Australia) of recent theoretical work on solvent effects. He directed attention to the calculations of Polansky on the interaction between two H atoms which predict a red shift in the atomic spectrum beyond a critical distance and a blue shift at closer distances, to the calculations by Longuet-Higgins and Pople of the red shift in the spectra of non-polar solutes in non-polar solvents arising from dispersive forces, and to McRae's formulations of the case of polar solute and polar solvent. The McRae formula predicts a frequency shift between absorption and fluorescence arising from the change of dipole moment between the ground and excited states, thus providing a method for measuring the dipole moments of excited states for comparison with calculated values.

One set of contributed papers in this section dealt with the spectra of aromatic hydrocarbons. Drs. G. R. Hunt and I. G. Ross (Physical Chemistry Department, University of Sydney) discussed a vibrational analysis of the 7000 A. and 3500 A. absorption systems of azulene which appears to confirm the predictions of Pariser and of Moffitt concerning the nature of the excited states. Dr. L. E. Lyons and Mr. G. C. Morris (Physical Chemistry Department, University of Sydney) presented results on the absorption of anthracene vapour (38,000 to 60,000 cm.$^{-1}$). They confirmed the second π–π transition as allowed and also observed four members of a Rydberg series converging to an ionization potential of 6·81 eV. Dr. N. S. Ham (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) reported some calculations by the free-electron model, with electronic interaction, of the spectrum of the perinaphthenylium cation $C_{13}H_9^+$, which agree well with the reported spectrum and also predict an unreported absorption band at about 600μ.

Studies by Dr. I. G. Ross and E. J. Wells (Physical Chemistry Department, University of Sydney) on the interesting tetrahedral molecules OsO_4 and RuO_4 failed to reproduce the extensive fine structure reported in the room temperature spectra by Langseth and Qviller in 1934. The authors gave a vibrational analysis of their spectra and used the energy-level scheme of Ballhausen and Liehr to assign the two allowed transitions. A theoretical paper by Dr. E. G. McRae (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) was concerned with electronically excited states of aggregated identical molecules; the intra-molecular vibrations were explicitly included. Two limiting cases were recognized, depending on whether the interaction energy was large or small with respect to the vibrational energy. An interpretation of the *J* band of N,N′-diethyl-pseudo-cyanine was offered on the basis of this theory.

Dr. H. A. McKenzie (Division of Food Preservation and Transport, Commonwealth Scientific and Industrial Research Organization) spoke on the difference spectra in acid solutions and in urea solutions of bovine serum albumin, ovalbumin and conalbumin.

Dr. L. E. Lyons (Physical Chemistry Department, University of Sydney), in opening the session on solid state spectroscopy, described a number of the effects

observed in ionic solids and semi-conductors. Such effects include structure in the absorption edge of the conduction band, hydrogen-like absorption series due to excitons, the high intensity of the conduction band absorption of germanium due to the low effective mass of the electrons, intervalence band transitions in germanium, and the magnetic splitting of the conduction-levels in InSb. The use of cyclotron resonance in determining the presence of excitons and in evaluating effective masses was also noted. Crystal field effects in the spectra of inorganic complexes and the Davydov splitting in molecular crystals were also discussed.

Dr. J. A. Friend (Chemistry Department, University of Tasmania) and Dr. Lyons have identified two transitions in the crystal spectrum of sodium nitrate, an allowed one at 2000 A. and a weak n-π transition at 2870 A. Dr. J. Ferguson (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) has analysed the polarized spectra of microcrystals of some cobalt (II) tetrahedral and octahedral complexes. The absorption spectra of the tetrahedral complexes of the type $CoPy_2X_2$ are modified by intermolecular interaction in the crystal while the octahedral complexes can be interpreted by simple crystal field theory. Mr. J. E. A. Alderson (Physics Department, University of Western Australia) discussed the luminescence spectra of thallium-doped potassium iodide in the spectrum range 600 A. to 2500 A. recorded on a normal incidence grating spectrometer with a photomultiplier as detector. Dr. L. E. Lyons, Dr. J. R. Walsh and Mr. J. W. White (Physical Chemistry Department, University of Sydney) presented the polarized visible spectrum of single crystals of phthalocyanine. An attempt was made to calculate the crystal spectrum using the Davydov theory, but the error in estimating the dipole vector is too large to allow an unambiguous assignment of the crystal levels.

Two papers on vacuum spectroscopic technique were presented. One by Mr. R. S. Crisp (Physics Department, University of Western Australia) described a grazing incidence, photon-counting grating spectrometer, working in the range 40–1000 A. It has been found that the soft X-ray band emission spectra obtained with this instrument change with the material of the grating (aluminium to glass), with time, and with the order in which the grating is used. These effects have no explanation at the moment. J. V. Sullivan (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) described a double-beam photoelectric 1 m. grating spectrometer, which has been tested down to 1500 A. The monochromator employs a concave grating in a new type of mounting in which deviations from the Rowland circle lead to no detectable loss in resolution. The double-beam system involves grazing incidence reflexion to split the exit beam and two electronically coupled photo-multipliers to record the spectra. Examples of spectra recorded over the range 1500–6000 A. were presented.

The second day of the conference began with a review of some of the infra-red, Raman and microwave work of 1958 by Prof. A. N. Hambly (Canberra University College). Topics discussed included: accurate determination of molecular geometries from gaseous Raman and microwave spectra; information on torsional modes barrier heights, barrier tunnelling and rotational isomerism from spectra in the cæsium iodide and microwave regions; pressure-induced transitions in gases. Perturbation effects and solvent effects need further study, for the reviewer pointed out that despite considerable work on the subject, the intensities of infra-red bands in polar solvents still do not accord with the theories.

In a research paper Prof. Hambly and J. G. Allpress (Chemistry Department, University of Melbourne) showed that infra-red spectra can be used to follow some solid state reactions. They illustrated their remarks by showing spectra of the products of reaction between U_3O_8 and alkali or alkaline-earth halides. They could detect differences that were not discernible in X-ray powder patterns.

Dr. E. Spinner (Australian National University, Canberra) discussed the vibration spectra and structure of the hydrochlorides of urea, thiourea and acetamide. Infra-red and Raman spectra suggest that the cations are formed by the addition of a proton to the nitrogen rather than to the oxygen atom and that the C—N bond in an amide is a pure single bond. Drs. N. S. Ham and J. B. Willis (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) dealt with the infra-red and Raman spectra of some isothiocyanates. They have identified the symmetrical NCS stretching frequency in —CH_2NCS types at about 670 cm.$^{-1}$, and at about 930 cm.$^{-1}$ in aromatic —NCS compounds. They also gave a detailed interpretation of the complex structure of the strong NCS characteristic band near 2100 cm.$^{-1}$. A. J. Costoulas (Department of Chemistry, University of New South Wales) gave a complete vibrational assignment for methyl-isothiocyanate. He agreed with Ham and Willis in the reassignment of the band at 676 cm.$^{-1}$ to the symmetrical NCS stretching frequency.

Prof. A. N. Hambly and R. H. Laby (Chemistry Department, University of Melbourne) discussed some criteria for allocating displacements of X—H bond stretching frequencies to the formation of hydrogen bonds. These are a double absorption band for weak intermolecular H-bonds and most weak intramolecular H-bonds, a double absorption band at low concentration in non-polar solvents for stronger intermolecular H-bonds, and a large displacement of frequency but no doubling on dilution for strong intramolecular H-bonds. Some exceptions were illustrated by the spectra of some ortho-amino acetophenones and anthranilic esters. The stable conformations of a series of methyl-*cyclo*-hexanols were reported by Dr. A. R. H. Cole and G. T. A. Müller (Chemistry Department, University of Western Australia). Small but significant differences in the stretching frequencies of axial (3627–32 cm.$^{-1}$) and equatorial (3622·5–23 cm.$^{-1}$) hydroxyl groups were used to determine the conformations. The methyl group was found to have a greater tendency to be equatorial than the hydroxyl group. A. G. Moritz (Department of Organic Chemistry, University of Adelaide) has examined the overall appearance of the 3000 cm.$^{-1}$ region in the infra-red spectra of a number of methyl-substituted polycyclic hydrocarbons. He has confirmed the general applicability of the correlation between the pattern of this region and the free valence number of the position of substitution in the parent hydrocarbon, a correlation first observed by Fuson and Josien for the methyl-1 : 2-benzanthracenes.

The results of a normal co-ordinate analysis of the 33 planar vibrations of naphthalene were presented by D. E. Freeman and Dr. I. G. Ross (Physical

Chemistry Department, University of Sydney). An iterative procedure of adjusting the force constants was used to force agreement with seven of the frequencies the assignment of which is most certain. This necessitates some reassignments, and the resultant interpretation is in general agreement with the work of Luther.

The simplest molecule discussed at the conference was hydrogen deuteride the weak dipole vibration spectrum of which was the subject of a paper by Dr. R. A. Durie (Coal Research Station, Commonwealth Scientific and Industrial Research Organization). The internuclear distance and the anharmonicity constants were calculated from measurements of the rotational structure of the (1,0), (2,0), and (3,0) vibration bands. Dr. D. W. Posener (Division of Electrotechnology, Commonwealth Scientific and Industrial Research Organization) presented the only paper on microwave spectroscopy. He has measured the electric field gradients at the oxygen and hydrogen nuclei in HDO and D_2O and has begun a measurement of the magnetic hyperfine splittings.

The following papers which were generally concerned with spectrographic apparatus and methods were also presented. Dr. H. Gollnow (Mt. Stromlo Observatory, Canberra) described a precision photoelectric setting device (accuracy about $0{\cdot}5\mu$) for the measurement of diffuse spectral lines. A fast-scanning infra-red ($1–5\mu$) spectrometer equipped with a cooled lead–tellurium detector was described by Dr. J. Tregallas-Williams (Defence Standards Laboratories, Maribyrnong). Another paper from the same laboratories, by G. L. White, discussed the application of polynomials fitted by 'least squares' methods, instead of the Friedel–McKinney equation, for the calibration of infra-red spectrometers. Some molecular spectra excited by shock waves were shown by Dr. C. L. Cook (Weapons Research Establishment, Salisbury), and the part played by a photographic spectrograph in routine control in the steel industry was outlined by J. H. Savage (Australian Iron and Steel, Port Kembla). Another paper on emission spectroscopy was given by J. M. Nobbs (Defence Standards Laboratories, South Australia), who described some of the effects of pulse shape on the spectral characteristics of pulsed discharges. He pointed out that the exponential shapes commonly used yielded lower sensitivities than most other shapes.

The final morning session of the conference took the form of a small symposium on atomic absorption spectroscopy, with a review and five research papers on the subject. A. Walsh (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) reviewed the present status of emission and atomic absorption spectroscopy. Recent advances in emission spectroscopy include better sources, the use of photomultipliers and the availability of vacuum instruments. The use by Margoshes and Scribner of a plasma-jet as a spectroscopic source was a new development which will be followed with great interest. However, the need for standards remains. This results essentially from three types of interference, namely, chemical, radiative and excitative. Atomic absorption spectroscopy avoids two of these but not the chemical interferences. In the opinion of the reviewer the value of atomic absorption spectroscopy has been established and it seems that any analysis that can be done by flame emission methods using atomic lines can be done as well and often better by atomic absorption methods.

J. E. Allan (Department of Agriculture, Hamilton, New Zealand) described the analysis of magnesium by atomic absorption. The measurement is carried out simultaneously with flame emission analyses for calcium, sodium and potassium, the other major cations present in plants and soils. The magnesium determination can be performed with the same ease as, and probably with greater reliability than, calcium, sodium and potassium determinations. The minor elements zinc, iron and manganese have also been determined by atomic absorption directly on plant ash solutions or soil extracts, but copper at present requires prior extraction with an organic solvent. D. J. David (Division of Plant Industry, Commonwealth Scientific and Industrial Research Organization) spoke about some of the chemical interferences encountered in applying atomic absorption to the analysis of plant material and soil extracts. In plant material analyses, he finds no interference for zinc, iron, copper and magnesium but he finds that calcium absorption is depressed by phosphate, aluminium and silicate. This interference is eliminated with 0·6 per cent magnesium ions and 2 per cent, volume/volume, sulphuric acid. With soil extracts the atomic absorption analysis is straightforward for sodium, potassium and magnesium, but in this case the interference with calcium is controlled by 0·15 per cent strontium ions. These two papers showed that many analyses important in plant and animal nutrition can be carried out successfully by the atomic absorption method.

Dr. J. B. Willis (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization) discussed the determination of blood serum calcium and magnesium levels by atomic absorption. Large quantities of strontium ions or the disodium salt of ethylene diamine tetracetic acid are needed to overcome the interference of phosphate and protein. Duplicate measurements for calcium and magnesium can be made directly on about 0·25 ml. of serum diluted 10–20 fold. Dr. P. Brownwell (Botany Department, University of Adelaide) is interested in analysing for very small quantities (about 0·01 p.p.m.) of sodium, which is a micronutrient for *Atriplex vesicaria*. The flame photometric results are misleading when the calcium concentration exceeds 0·02 *M*. The atomic absorption method using '*Si-ro-spec*', a simple instrument designed by Box and Walsh for this purpose, at present enables accurate measurements of about 1 p.p.m. sodium in the presence of large excesses of calcium (4 *M*) and potassium.

The final contribution, by Dr. B. M. Gatehouse and Mr. A. Walsh (Division of Chemical Physics, Commonwealth Scientific and Industrial Research Organization), was concerned with the direct analysis of metals and alloys. By sputtering from a metal surface in the presence of about 1 mm. mercury pressure of neon they were able to obtain a calibration curve and to determine 1 p.p.m. silver in a copper–silver alloy. They suggested that this method may be suitable for elements such as aluminium and boron which do not give atoms in the flame.

In a concluding discussion members agreed unanimously that a standing committee should be set up to organize similar conferences at regular intervals.

NORMAN S. HAM

PROTEIN BIOGENESIS

A COLLOQUIUM on "Specificity in Protein Biogenesis" was held in Louvain during June 8–9 by the Centre interuniversitaire de Recherches enzymologiques, a government-sponsored association grouping several Belgian biochemical laboratories. The aim of the organizers was to bring together scientists actively engaged in research on protein synthesis and to establish new contacts with the Belgian groups interested in this field. The meeting was arranged on an informal basis and the speakers were asked to discuss freely the current work of their laboratories; the lectures will not be published.

Present ideas on the relationship between deoxyribonucleic acid and protein structures were lucidly summarized and discussed by C. Levinthal (Massachusetts Institute of Technology). Since deoxyribonucleic acid and protein are both linear polymers, it would seem that a simple relation should exist between the arrangement of the amino-acids in the protein and that of the nucleotides in deoxyribonucleic acid of the corresponding gene—probably a point-to-point correspondence between the two sequences. Some of the characteristics of the coding system serving to translate one sequence into the other can be deduced from what is known about deoxyribonucleic acid and protein structure. It seems feasible at present to check experimentally the idea of collinearity between the structure of the two types of polymers. Find an organism in which genetic maps can be established with great accuracy, choose an enzyme molecule of moderate size produced by this organism and devise a good selection principle for recovering the useful mutants. Isolate several mutants of the genetic locus of the enzyme, map the mutation sites, isolate the abnormal proteins, if any, which are produced in place of the normal enzyme by the mutants, and locate the differences within the protein molecules. This gives a test of the correspondence between deoxyribonucleic acid and protein fine structure.

C. Levinthal described the progress of his own work on the genetic control of the formation of a phosphatase in *E. coli*. Thirteen different mutants resulting from mutations within the locus of the phosphatase have been isolated so far; the linear order of the mutation sites has been established by a very interesting use of the transfer of genetic material during bacterial conjugation. Unfortunately only one of the thirteen mutants produced a recognizable modified phosphatase protein. Both the normal and the abnormal enzymes have been isolated, and their structure is now being studied. Future developments along this line of research will be watched with great interest.

In bacterial systems, genetic analysis is ahead of chemical knowledge. In man, on the other hand, where genetic analysis is at a great disadvantage, brilliant success has been achieved on the chemical side. J. Hunt (Cambridge) reviewed the main results obtained by the Cavendish Laboratory group on the structure of abnormal hæmoglobins. This work will undoubtedly become a classic of genetics and biochemistry. Hæmoglobins *S* and *C* differ from the normal protein in only one detail: the replacement of one glutamic acid in the β chain by valine or lysine respectively. Hæmoglobin *E* differs from normal hæmoglobin by the replacement of another glutamic residue by lysine; in hæmoglobin *G*, still another glutamic is replaced by glycine. Thus genetic differences presumably arising from mutations can result in the replacement of individual amino-acids at specified places in the polypeptides.

J. Hunt further described in detail his recent work on fœtal hæmoglobin. He has shown that one of the two polypeptide chains of fœtal hæmoglobin (chain α) is identical with that of normal adult hæmoglobin. This discovery opens new perspectives and will certainly give important information on the mechanism of the genetic control of protein structure, for here is a simple case of differentiation at the molecular level. The structure of fœtal hæmoglobin of infants carrying genes of abnormal adult hæmoglobins will be very informative. It might indicate whether the switch from fœtal to adult hæmoglobin during development results from the inhibition of the activity of a gene and the unveiling of another gene, or whether the change occurs somewhere between the gene and the protein-making system and consists in a change of expression of the same gene.

Other aspects of the control of the synthesis of specific proteins were discussed by B. Magasanik (Harvard University), who considered the phenomena of enzyme induction and repression and the function of ribonucleic acid in the synthesis of bacterial protein. B. Magasanik reported results obtained in his laboratory on various mutants which require amino-acids, nucleic acid precursors, or certain energy sources. By a very ingenious use of these mutants, several aspects of the correlation between ribonucleic acid content and level of protein synthesis were checked. Increased rate of protein synthesis goes together with an increased content of the bacteria in both soluble ribonucleic acid and ribosome ribonucleic acid. This is comparable to the well-known relation found in higher organisms. Studies on ribonucleic acid and protein synthesis during adaptation, especially in 'diauxie' experiments, indicate that the synthesis of new ribonucleic acid does not accompany enzyme adaptation. The results are compatible with a catalytic function of ribonucleic acid in protein synthesis, and with the view that induction and repression of enzyme synthesis rest upon the control of the activity of pre-existing protein-forming centres, rather than on the formation or destruction of such systems.

The existence of specific ribonucleic acid molecules capable of carrying some sort of genetic information is established by the discovery that pure virus ribonucleic acid is able to cause infection. Present knowledge on the structure of tobacco mosaic virus was summarized by H. Fraenkel-Conrat (University of California), who presented results of his current work on the molecular size of the virus ribonucleic acid. End-group determination in the virus acid by a combination of tracer methods with specific enzymic degradation and controlled chemical oxidation indicates that each virus particle might contain one single molecule of ribonucleic acid made up of some 6,000 nucleotides in one chain. The polypeptide chain of the virus protein contains only about 150 amino-acid residues. It would seem, therefore,

that the ribonucleic acid of the virus is large enough to carry much more information than that which is required for controlling the primary structure of the protein unit contained in the finished virus. This raises several problems for future research. H. Fraenkel-Conrat also reported very interesting results on a special state of the virus ribonucleic acid at the beginning of infection.

Another approach to the specific function of ribonucleic acid in protein synthesis is the artificial modification of the structure of the acid. This has been done by chemical means in the case of virus ribonucleic acid. In bacteria, composition of the acid can be changed by growing the organisms in the presence of analogues of the normal purines or pyrimidines. F. Gros (Institut Pasteur, Paris) gave a very clear account of research on the effects of fluorouracil on protein synthesis in *E. coli*. Incorporation of all the individual amino-acids does not respond in the same way to fluorouracil. For example, the incorporation of proline and tyrosine is depressed whereas that of arginine is stimulated. These changes appear to reflect qualitative as well as quantitative modifications in the protein equipment of the organism, indicating that the analogue may actually interfere with the agents which control protein structure. A phosphatase formed in the presence of the analogue has a normal enzymic activity although it contains less proline than the normal enzyme; thus it is probably slightly modified at a place which is not important for the catalytic properties of the protein. On the contrary, β-galactosidase synthesis is abolished and replaced by the formation of some related inactive protein. It is striking that fluorouracil specifically reduces the fixation of proline and of tyrosine on soluble ribonucleic acid at the same time as it reduces the incorporation of these same amino-acids into the proteins. This indicates that soluble ribonucleic acid plays an important part in the specificity of protein formation. These results also support current views according to which activated amino-acids are bound to soluble ribonucleic acid before condensing into polypeptides.

T. Hultin (Wenner Gren Institute, Stockholm) reported observations on animal tissues which indicate that another pathway of amino-acid incorporation might exist beside that passing through soluble ribonucleic acid. That the latter must also be operative was shown by several of his results which agree with the classical scheme. However, he obtained, by means of new techniques of isolation of ribosomes and by fractionation of supernatant preparations, a system in which amino-acids are incorporated into proteins in the particles in the absence of soluble ribonucleic acid. A protein of the supernatant is required for incorporation with this system. Protein synthesis which can occur in isolated mitochondria or nuclei appears to depend on the presence in these cell organelles of particles closely resembling the ribosomes of the cytoplasmic ground substance.

Of great benefit to the meeting was the presence in the audience of biochemists from different countries who had taken part a few days before in Brussels in the Solvay Conference on Nucleoproteins. All the lectures were followed by very good discussions. The meeting was closed by a general discussion which concerned the function of the various ribonucleic acid fractions, the transfer of information from gene to protein, and coding problems.

H. CHANTRENNE

PROGRESS IN GAS CHROMATOGRAPHY

AN informal symposium of the Gas Chromatography Discussion Group (associated with the Hydrocarbon Research Group of the Institute of Petroleum) was held at the University of Bristol on September 25 under the chairmanship of Mr. C. S. G. Phillips.

Dr. F. H. Pollard commented on the enthusiasm and free interchange of ideas among workers in this field which was undoubtedly responsible for the present advanced state of the art. Having regard to the success achieved by him in the field of inorganic separations by paper chromatography, it was not surprising that he should mention the possible separation of such materials by gas chromatography.

The outstanding feature of the meeting was the demonstration by Mr. R. P. W. Scott of the presentation of gas chromatographic data with a high-persistence cathode ray tube. With capillary columns it is possible to effect separations at speeds much greater than the response of conventional recorders, and in order to take full advantage of the technique in its application to kinetic and other studies a means of high-speed recording is essential. Mr. Scott, using a 70-ft. column, demonstrated separations of 100° C. boiling-range samples in less than 1 min. with his apparatus which, inclusive of automatic repetitive sample injection system, cost less than £80 for materials.

The discussion which followed a paper by Mr. C. L. A. Harbourn on quantitative determinations showed that this aspect of the subject is one that affects most users of the technique. As yet, however, if one uses the published literature as a guide, it would appear to have received very little attention. The well-prepared paper covered methods of peak measurement, sources of error and repeatability of calibration, internal standard, and normalization methods, and interpretation of unresolved peaks. Recent developments in integrators and the use of analogue computers and tape recorders were also discussed.

Some of the practical aspects of the measurement of retention volumes were dealt with by Dr. G. W. A. Rijnders, and Dr. C. R. Patrick mentioned some of the problems attached to 'scaling-up' analytical columns to sizes capable of handling up to 10-gm. samples. The values of height equivalent of a theoretical plate (H.E.T.P.) increase and the much higher volumetric flow-rates necessitate modifications to the design of hot-wire detectors. Mr. D. H. Desty read a paper by Dr. J. Janak, who unfortunately was unable to be present, describing the application of gas chromatography to the identification of structure of involatile substances by pyrolysis and subsequent analysis of the products.

Members attending, among whom were some from the United States and Europe, were able to inspect and see working a good selection of the commercial instruments now available for laboratory and process control work.

C. G. SCOTT

ORIGIN AND DISPERSAL OF FLOWERING PLANTS

A TOTAL of no less than thirty-nine papers presented at the Montreal Ninth International Botanical Congress during August 19–29 dealt directly or indirectly with the origin and dispersal of flowering plants, providing a modern commentary on these two long-standing fundamental questions.

A review of the evidence for a pre-Cretaceous origin was given in two invited papers before a joint symposium of the palæobotany and morphology and anatomy sections. In the first of these A. J. Eames, discussing morphological testimony for a Palæozoic origin, maintained that studies in the Ranales showed that the primitive flower was bisexual and beetle-pollinated, that unisexuality and diœcism accompanied by insect pollination, then anemophily and the elaboration of simple sporophylls, were major advances which must have required long periods of time. These steps had already been taken before the Lower Cretaceous. Eames suggested that the search for ancestral angiospermous stock might again be directed towards the late Palæozoic ferns, even possibly the homosporous ones. Besides the evidence from comparative anatomy, there was direct evidence in the form of pollen, wood and leaf impressions from the Triassic and Jurassic.

L. Van der Pijl, opening the Darwin Centennial Symposium, considered that the angiosperm flower must have differentiated in contact with beetles, already present in the Permian, whereas insects are not common until the Cretaceous. Persisting traces of beetle pollination, he pointed out, are found in extant groups. V. Grant, in an account of the diversity of pollination systems in the Polemoniaceae, appeared largely to concur with Van der Pijl's view of the primitive flower. In different papers, A. S. Foster described how certain herbaceous ranalean forms might represent an early angiospermous type with dichotomous venation; W. N. Stewart proposed that Conrad's monophyletic classification of vascular plants be revived, despite the recent trend towards a polyphyletic one.

The difficulties inherent in the discovery of palæobotanical evidence of angiospermous origins were illustrated by N. H. Hughes, who demonstrated Jurassic probably gymnospermous pollen, and by W. G. Chaloner, who described how carboniferous plants with spores which could be found occurring commonly could nevertheless be poorly represented as macro-fossils. On the other hand, in a paper on Palæozoic marine sequences from New York and Australia, W. R. Evitt further documented the antiquity of vascular plant stocks.

In the second invited paper of the first symposium D. I. Axelrod dealt primarily with a third line of evidence, namely, modern distribution patterns. He illustrated with tables the preponderance of families entirely or largely tropical or with a large tropical section. Basic distribution patterns which emerged were tropical; tropical and extra-tropical, boreal or austral; dry and temperate derived. Such evidence, Axelrod considered, favours the supposed existence of angiosperms in the later Palæozoic in upland tropical regions where they underwent quantum evolution.

Many biosystematic, palynological and palæological papers dealt to a greater or lesser extent with flowering plant distribution. E. Hulten, opening a symposium on alpigenous and arctogenous plants with specific illustrations of various types of circumpolar evolution, pointed out that increasing knowledge of this kind provided further material for Quaternary phytogeography. W. A. Weber directed attention to the southern survival of many arctic and sub-arctic species, more especially in the Colorado Rocky Mountains. E. Dahl, considering amphi-Atlantic arctic-alpine plants, discussed evidence for their migration across a Pliocene or Pleistocene land-bridge. Dahl suggested that the West Arctic Scandinavian species survived the last ice-age in coastal refugia rather than in Central Europe. A. Tolmatchev, in an abstract, stated that the Arctic flora was first formed in the Pliocene–Pleistocene, from indigenous elements in the treeless areas on mountain summits and slopes and similar open habitats. T. W. Böcher held that most arctic or montane species groups are probably of Tertiary origin, but that considerable speciation occurred within these groups in the Quaternary, supported by geographical or genetical barriers. J. A. Calder, discussing post-Pleistocene distributions in British Columbia, suggested that extensive refugia had existed in Alaska, the Yukon, also in the mountains of west Mackenzie and the Queen Charlotte Isles during the period of maximum glaciation.

On modern montane distributions, K. O. Hedberg described the Afro-alpine flora as a complex derived from Mediterranean, Himalayan, South African and Temperate, besides Tropical African floras, and which had probably existed since the Miocene. J. K. Morton found a higher incidence of polyploidy in the Cameroons mountain flora than in lowland Ghana; I. Manton, in a presentation otherwise restricted to ferns, referred to this Cameroons flora as an "island" one, comparable with that of Ceylon in respect of the occurrence of polyploidy. C. Favarger described how the incidence of polyploidy in Switzerland in certain alpine groups coincided with a floristic subdivision. G. S. Puri considered that during the Pleistocene glaciation the Himalayas were invaded by conifers, so that pure conifers or oak-conifer seral stages may now be found there alongside oak climaxes. In an abstract B. Pawlowski stated that there could have been many survivors of an endemic Tertiary flora on the east and some on the west of the Carpathians. P. Gorchakovsky similarly described how, apart from endemics, the Ural flora was composed of relics from pre-Glacial broad-leaved forests and glacial ones, from mountain steppe of Asiatic origin and from Pleistocene high mountain relics of Asiatic and of Siberian origin.

Dealing with restricted aspects of modern temperate floras, A. J. Sharp thought that much of the flora of the newer coastal plains of Tennessee and the glaciated terrain to the north were segregates of a more ancient flora of the Southern Appalachian highlands. L. Laasimer divided the flora of Estonia into East Baltic, related to eastern Europe and West Baltic to Central Europe, respectively. A. A. Beetle discussed how, in the southern part of central Wyoming, differences in grass distribution were due to differences in the age and origin of the various species. In the Cretaceous, the Tethys Sea, by dividing the Old

World climates, established contrasts between the predominantly tropical panicoid and the predominantly temperate festucoid grass types. Describing the phytogeography of Chinese species of *Fraxinus*, Shiu-Ying Hu stated that one species of a primitive group extended to Sumatra, two species from west and central China have close affinities with north American species, one from north China and one from Yunnan are close to European species.

For tropical floras, A. Liogier held that Cuba, although tropical, drew most of its species from subtropical and temperate North America. The present Cuban distributions suggest that the bulk of the flora came chiefly from South America through Central America during the Mesozoic and Cainozoic, when Cuba and Jamaica were united with Honduras. L. B. Smith described how the flora of south Brazil had its greatest source in central Brazil, there being continuous linkages with the Amazon, Andes and Argentine, discontinuous ones with the Amazon, North America, the Andes and Africa. The mountainous and the low-lying floras of tropical America were considered by F. Miranda to have had a greater diffusion in past Tertiary periods. These floras were partially dominated later in tropical zones by a more macrothermic flora, and almost eliminated in North America by a more microthermic one.

Papers were provided by K. A. Chowdhury, E. Dorf, W. L. Fry, J. Gray, T. Jimbo and K. Rasky, describing angiosperms in various Tertiary floras of India, Wyoming, Canada, Oregon, Japan and Hungary respectively. C. A. Arnold and J. S. Lowther and J. F. Grayson and R. L. Pierce illustrated the low representation of angiosperms in two American Cretaceous floras. L. A. Kuprianova described how palynological studies suggested that *Liquidambar* already occurred in the Upper Cretaceous, that it is not a typical representative of the Arcto-Tertiary flora, and indicated ancient floral relationships between the Mediterranean, Central America and Eastern Asia. In a paper to the Taxonomy Section, J. Ewan reminded botanists that zoogeographical data may prove illuminating in the interpretation of existing floristic patterns, citing examples including that of the wood rat which persists together with the relict Sierra Madrean flora on outlying Rocky Mountain summits.

A. S. BOUGHEY

THE NATIONAL HEALTH SERVICE IN BRITAIN

THE Acton Society Trust has produced a valuable survey of the National Health Service in Britain prepared by Sir George Schuster*. Part of the report is a discussion of the general problems of leadership in a State service; this has been dealt with elsewhere (see *Nature*, October 10, p. 1087).

Sir George then returns to the National Health Service itself. He starts with two personal observations. With keen appreciation of the achievements of the National Health Service in Britain in these first ten years, and the belief that too little was far better than too much interference from the central authority, he believes that the time has come for a general overhaul and tuning-up of the administrative machine, for an interpretation of the lessons to be learned from the practical experience of these first ten years, and for a new effort, in the light of this practical experience, to understand what is required to ensure that the National Hospital Service can meet the true needs, individual and social, of the people of Britain in these times. Secondly, writing as one desperately anxious to see the best use made of this fine Service, he emphasizes the immense burden of current routine work now falling upon the senior staff of the Ministry. This has left them with far too little time to attend to many of the things he suggests need to be done.

This matter of overburdening the senior officials is not confined to the Health Service, and although its seriousness has often been admitted since the War, there is little indication that much has been done to relieve the situation. All these, however, are matters that could bear closely on the work of other sections of the public service, where the administration of highly technical matters and the control of technical and professional staffs are involved. It is to such major issues that Parliament might well have been expected to give its attention; but apart from Sir Keith Joseph's reference to the need for a research or planning staff in the Ministry, they were unnoticed in the House of Commons debate on the National Health Service on July 15.

It is the quality of Sir George Schuster's thought and the character of his approach rather than his particular proposals that are of most general interest. While he has always conceived of the hospital service as a great fighting service, in which the heads of hospital boards should be entitled to expect from the Ministry something like the leadership and direction which army commanders would get from their commander-in-chief and his general headquarters, he stresses the paramount importance of keeping the hospital service human. Inadequate leadership, inefficient management, muddled organization can destroy all possibility of the workers in the service getting satisfaction from their work in a manner that keeps alive their enthusiasm and fulfils a sense of vocation. This, however, is an essential element in providing what the patients themselves require above all—efficient curative treatment—and Sir George is well aware that good organization is only a means to an end and he reminds us that in the widest sense the welfare of the patients means taking into account their comfort, peace of mind and all the psychological and social implications of stays in hospital.

Sir George does not devote space to the consideration of these issues. He is content to note them among the great needs and opportunities in this service which call for a combination of hard-headed administrative efficiency, highly developed working skills, single-hearted pursuit of scientific knowledge and human kindness. There are other fields in which the demands are no less, and even if in them it might be too much to assert that without human kindness the true purpose and value of the work must fail, without it full success or true efficiency are hard to achieve.

* Creative Leadership in a State Service: a General Survey. (Hospitals and the State. Hospital Organization and Administration under the National Health Service, Sixth and Final Research Paper.) Pp. iii+80. (London: Acton Society Trust, 1959.) 4*s*.

Sir George believes, however, that a Government department can function as the directing authority of a great operating service like the Hospital Service in Britain, and the vast expenditure involved could not and should not be removed from the direct control of a Minister responsible to Parliament. If, however, the Hospital Service is to remain the direct responsibility of a Minister and a Government department, Sir George considers that more attention must be given to the staffing of that department and to the arrangements for liaison between it and the decentralized formations, and that a central intelligence organization must be provided. Further, he suggests that on all three points lessons can be learned from the experience of other Government departments which have responsibilities in relation to operating services.

On the first point, he does not see how the Ministry can give the understanding leadership which is required unless the highest posts on its staff are held by officers who have had practical experience of hospital administration at operational level, and in support of his argument he quotes the staffing arrangements of the Fighting Service Ministries and of the Colonial Office. Sir George pays tribute to the value of the work done by many officers appointed before such experience was available, and he does not advocate immediate wholesale change but rather exchanges of staff on the lines of the Colonial Office arrangements. His stress is laid on the quality of the officers available to fill the various posts and the need in the National Hospital Service for new administrative skills, for which there is at present no readily available source of supply. This last, as he recognizes, is a need which often occurs elsewhere to-day as a result of modern development, and the Hospital Service must take deliberate action to meet this need as private enterprise has done in the field of industry.

It might be observed here that if this need for statesmanship is to be met in the Health Service, in industry, or elsewhere, those who meet that need must be treated as statesmen. There is no place for the type of party politics which seeks to misrepresent opponents or those who may be entrusted with the execution of schemes or policies which are not in line with party doctrine. It is significant that in supporting the Acton Society Trust's proposal that the Minister in charge of the Health Service should have a seat in the Cabinet, Sir George stresses the danger of frequent change of office, and his own proposals would make the chief permanent official in the Ministry a professional man.

This does not mean that Sir George is here advocating that the expert should be permanently at the top; but his suggestion goes far beyond the claim that the expert should be considered for the highest administrative posts. It implies that the expert by virtue of his expertise is particularly suited to supply the type of specialized administration needed. He must, of course, possess administrative capacity: there is nothing in Sir George Schuster's pamphlet to countenance incompetence in management or administration, but much to stimulate more thought about the way in which to meet the need for administrators and the type of training and experience they should receive.

On the second point, that of liaison between the Ministry and the hospital authorities, Sir George suggests that there are lessons to be learned from the best traditions of the inspectors of schools under the Ministry of Education; but here again Sir George pleads for constructive thinking and refrains from specific proposals. His stress on joint consultation at the centre and once again on the quality of the liaison officers clearly has implications far beyond the Health Service, and his strongest criticism is reserved for the perfunctoriness with which liaison is often treated. Positive measures and constructive thought are always required to provide an effective two-way flow of ideas.

Sir George Schuster's major proposal is in regard to his third point and, in line with a main recommendation of the Guillebaud report, he recommends the establishment of a central intelligence and statistical department. This, however, should be an integral part of the executive, with its own creative role as a detector of problems and a productive source of wisdom. Sir George has in mind something on the lines of the Office of Special Enquiries and Reports under the direction of Michael Sadler at the Ministry of Education, equipped with an intelligence staff which would keep under constant review the development of hospital practice, in its social as well as its medical setting, in Britain and other countries, and which would publish a series of reports which might be accepted throughout the world as authoritative. With such a staff the Ministry could not only carry out its own investigations, but could also provide valuable stimulants to work by the hospital authorities. Particularly in its social aspects, the research here required needs central guidance and co-ordination, and Sir George's own experience as chairman of a regional hospital board has convinced him of the great flow of evidence of clinical, human and social interest which requires recording, co-ordination and interpretation.

Beyond this Sir George points to the need for creative thought, such as demands the services of men of wisdom and comprehension, combined with knowledge of medical affairs. How to produce such men and women is one of the real challenges which this rapidly changing world makes on professional organizations to-day, and there is much indeed in Sir George Schuster's comments that deserves careful study by professional men and women of professions other than that of medicine. So far as the National Health Service in Britain is concerned, Sir George Schuster's investigation suggests that this is very seriously understaffed as regards first-class administrators in comparison with large industrial organizations, and some improvement here may well be the first step required to implement his more specific proposals to remedying the alleged weakness of the voluntary committee system and to promote the intensive expert study of the hospital cost structure, the full use of efficiency techniques by the hospital authorities, including the introduction of work-study methods, and the application of operational research to some of the problems of the hospital service.

These last are clearly proposals limited specifically to the Hospital Service and to some of them an interested group of members of Parliament is already giving attention. The Minister of Health has stated that the Ministry's present experimental organization and methods unit is to be enlarged and made permanent, and that he has accepted the offer of a group of management consultants to undertake a series of surveys, at their own expense, to demonstrate the economies and improvements in efficiency which could be achieved in the hospital service by work study.

PREVENTING JUVENILE DELINQUENCY

SEVERAL years ago the United States Children's Bureau, as part of its programme on juvenile delinquency, published a report about the effectiveness of measures in delinquency prevention. The analysis was based on evaluative studies conducted over the previous twenty-five years or so.

The review led to the conclusion that programmes for the prevention of juvenile delinquency had not been notably effective. This conclusion was tempered by two facts. First, few programmes, relatively speaking, had been evaluated, and most of those not adequately. Secondly, many of the evaluative studies were out of date since they dealt with programmes and methods that to-day might not be considered the best. Moreover, there were hints that good results had been achieved with certain types of delinquent children in certain circumstances. This was notably the case in child guidance work and was perhaps also true of the kind of neighbourhood work associated with the name of Clifford Shaw.

Some of the newer programmes and methods seemed, however, to give promise of more favourable findings. Among those mentioned in the report were various devices for 'reaching out' to youngsters and their parents with services they were unlikely to seek for themselves; for example, group work with delinquent and predelinquent gangs, case work or group work with 'resistant' families. These and other newly devised programmes seemed to be succeeding where older ones had failed and to be benefiting both from the experience of their predecessors and from recent advances in knowledge about human behaviour and motivation.

In an issue of the *Annals of the American Academy of Political and Social Science*, measures of delinquency prevention and their effectiveness are continued with reports from practitioners and research workers who have been closely associated with these efforts (**322**, March 1959).

The most striking change, according to Helen L. Wilmer, of the U.S. Department of Health, Education and Welfare, is in the level of sophistication shown in the reports. This is shown in the way the work with delinquent youngsters and their parents is carried on, in the psychological and sociological knowledge underlying the work, and in the methods employed in its evaluation. Much has been learned in all these areas in recent years. These articles show programmes of delinquency prevention both benefiting from that advance and contributing to it.

Perhaps the most important contribution of this series of articles lies in the picture it provides of the kinds of young people who are likely to become chronically delinquent and of the kinds of homes and neighbourhoods they live in. The picture is not a new one but is drawn in a way that reveals, more vividly than usual, the fears, the discouragement and the wish to be like other people that characterize these young people and their parents. The treatment measures described, both those that would improve the environment and those that are directed toward the individuals, take their direction from this knowledge. The knowledge itself is rooted both in the social sciences and in psychology and demonstrates their interrelatedness.

The articles deal with small programmes and, in part, short-lived efforts. Few of them report scientifically established results. Nevertheless, in their conception of what needs to be done and in their suggestions for some ways of doing it, they hold the hope that the problem of delinquency can be reduced if communities are willing to put the effort required into the work.

THE ORIGINS OF LOVE

PSYCHOLOGISTS, sociologists and anthropologists commonly hold the view that the infant learns to love through the association of the mother's face, body and other physical characteristics with the alleviation of internal biological tensions, particularly hunger and thirst. Psycho-analysts have tended to emphasize the importance of attaining and sucking at the breast as the basis for affectional development. Recently a number of child psychiatrists have questioned such simple explanations. Some argue that affectionate handling in the act of nursing is a variable of importance, whereas a few workers suggest that the composite activities of nursing, contact, clinging and even seeing and hearing work together to elicit the infant's love for his mother.

It is difficult, if not impossible, to use human infants as subjects for the studies necessary to break through the present speculative impasse. For several years a group at the Primate Laboratory of the University of Wisconsin has been using baby rhesus monkeys in a study that has begun to yield significant insights into the origin of the infant's love for his mother. A report has been prepared by Harry F. Harlow*.

The interest in infant-monkey love grew out of a research programme that involved the separation of monkeys from their mothers a few hours after birth. The investigators were impressed by the deep personal attachments that the monkeys formed for the diaper pads, and by the distress that they exhibited when the pads were removed briefly once a day for the purposes of sanitation. The behaviour of the infant monkeys was reminiscent of the human infant's attachment to its blankets, pillows or rag dolls. These observations suggested a series of experiments to compare the importance of nursing and all associated activities with that of simple bodily contact in engendering the infant monkey's attachment to its mother. Two surrogate mother monkeys were prepared. One is a bare welded-wire cylindrical form surmounted by a wooden head with a crude face. In the other the welded wire is cushioned by a sheathing of terry-cloth. Eight new-born monkeys

* *Scientific American*, 200, 6; June 1959.

were placed in individual cages, each with equal access to a cloth and a wire mother. Four of the infants received their milk from one mother and four from the other, the milk being supplied in each case by a nursing bottle, with its nipple protruding from the mother's 'breast'.

The two mothers quickly proved to be physiologically equivalent. The monkeys in the two groups drank the same amount of milk and gained weight at the same rate. But the two mothers proved to be by no means psychologically equivalent. Records showed that both groups of infants spent far more time climbing and clinging on their cloth-covered mothers than they did on their wire mothers. As the monkeys grew older, they tended to spend an increasing amount of time clinging to and cuddling her pliant terry-cloth surface. Those that secured their nourishment from the wire mother showed no tendency to spend more time on her than feeding required, contradicting the idea that affection is a response that is learned or derived in association with the reduction of hunger or thirst. These results indicate the importance of bodily contact and the immediate comfort it supplies in forming the infant's attachment for its mother; the cloth-covered mother surrogate is an eminently satisfactory mother.

The time that the infant monkeys spent cuddling on their surrogate mothers was a strong but perhaps not conclusive index of emotional attachment. Would they also seek the inanimate mother for comfort and security when they were subjected to emotional stress? With this question in mind the monkey infants were exposed to the stress of fear by presenting them with strange objects, for example a mechanical teddy bear which moved forward, beating a drum. Whether the infants had nursed from the wire or the cloth mother, they overwhelmingly sought succour from the cloth one; this differential in behaviour was enhanced with the passage of time and the acquisition of experience. All tests show that the infant monkey's relationship to its surrogate mother is a full one. Comparison with the behaviour of infant monkeys raised by their real mothers confirms this view.

While bodily contact clearly plays the prime part in developing infantile affection, other types of stimulation presumably supplement its effects. A search has been made for these factors. Western culture parents appreciate that rocking a baby or walking with him somehow promotes his psychological and physiological well-being. The responsiveness of infant monkeys to two cloth mothers, one stationary and one rocking, was now compared. All preferred the rocking mother, though the degree of preference varied considerably from day to day and from monkey to monkey. Motion does appear to enhance affection, albeit far less significantly than simple contact. The act of clinging, in itself, also seems to have a role in promoting psychological and physiological well-being.

Still other elements in the relationship remain to be investigated systematically. The warmth of the mother's body would appear to play its part in strengthening the infant's ties to the mother. Observations have not yet confirmed this hypothesis. Heating a cloth mother does not seem to increase the attractiveness of the mother to the infant monkey and infants readily abandon a heating pad for an unheated mother surrogate. Visual stimulation may forge an additional link. It is also possible that particular sounds and even odours may play some part in the normal development of response or attention.

The depth and persistence of attachment to the mother depend not only on the kind of stimuli that the young animal receives but also on when it receives them. Experiments with ducks show that imprinting is most effective during a critical period soon after hatching; beyond a certain age it cannot take place at all. From preliminary experiments with monkeys it has been found that their affectional responses develop, or fail to develop, according to a similar pattern.

NORTH–SOUTH ANISOTROPY AND ANTICIPATORY INCREASE OF INTENSITY ASSOCIATED WITH THE COSMIC-RAY STORM OF FEBRUARY 11, 1958

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THE time variations of cosmic rays have been measured during the International Geophysical Year with standard instruments at a large number of places on the Earth, and several studies have been made of the energy dependence of the primary variations and the anisotropy which is often associated with primary variations of intensity. From an examination of Forbush-type decreases, Fenton, Fenton and Rose[1] have come to the conclusion that the cause of the transient intensity decreases is variable in its energy dependence from a few BeV. to more than 30 BeV. The variation in response to transient decreases observed with similar equipment at different stations suggests that a primary anisotropy is present at these times. Lockwood[2] has examined the detailed structure of several Forbush-type decreases in the intensity of local neutrons during 1955–58. He finds that in most of the decreases there was a magnetic storm at the onset. Flare activity during the preceding 30 hr. was high and there was some indication of an intensity maximum during the 12-hr. period preceding the start of the decrease. He comments that such an anticipatory effect might be due to the albedo of the moving magnetic gas cloud, but that further results are

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Table 1. Chronology of Events associated with the Forbush Decrease of February 11, 1958
ΔC and ΔH respectively indicate the change of cosmic-ray intensity (CR) and of the horizontal component of geomagnetic field at Virginia

Date	U.T.	Solar event	U.T.	Terrestrial effect :	Cosmic ray features
9–2–58	0207	*2+ Flare		Radio fade-out	
	2053–2120	Type III and Type I radio bursts			
	2139	*2+ Flare E04.S20		Radio fade-out	
10–2–58	1325	*2+ Flare with major burst radio noise W67, S12		Radio fade-out	
			2100	CR maximum $+ \Delta C \approx +1$ per cent	(1) Anticipatory increase at equator only related to high energy
11–2–58			0120	S.C. storm	
			0126	$+ \Delta H, - \Delta C$	(2) Decrease starts at high latitudes
			0130	Aurora	
			0154	$+ \Delta H, - \Delta C$	
			0300	CR minimum	(3) 0300 minimum at equator
					(4) 0500 minimum in mesons
			0622	X-ray $\Delta H = 0$	
			0635	Maximum absorption, galactic noise of 18 Mc./s.	
					(5) 0700 minimum at high latitudes
			0730	X-ray ends	
			0850	X-ray and ΔH	(6) Increase commences
			1000	Maxima aurora	
			1100	CR maximum	Seen in instruments with high and low energy response. Not observed in stations in 120° E. belt, nor in the southern hemisphere

needed to substantiate any anticipatory effect. McCracken and Parsons[3] have made a very interesting analysis of a Forbush-type event which occurred on October 21, 1957. They found that there was a preliminary depression prior to the commencement of the Forbush decrease and they comment that it was not due to the arrival of solar matter at the Earth since it occurred before the magnetic disturbances. They conclude from studies made at several stations that the preliminary depression must be attributed to a cause located at some distance from the Earth, and since it is not observed simultaneously at all stations its explanation requires some rather special form of short-lived primary anisotropy. McCracken[4] has analysed the anisotropy of a number of Forbush-type decreases which were preceded by decreases. Yoshida and Wada[5] have directed attention to increases of intensity which occur after the onset of cosmic-ray storms. They believe that the increases are mainly isotropic and have an energy-dependence nearly the same as that of the decreases.

Table 2. Particulars of Cosmic-Ray Neutron Monitor Stations used in Analysis

Code	Station	Geog. Lat.	Geomag. Lat.	Long.	Investigator
A	Murchison Bay	80° N.	76° N.	18° E.	Dr. A. E. Sandstrom, Sweden
B	Churchill	59° N.	69° N.	94° W.	Dr. D. C. Rose, Canada
C	Leeds	53° N.	57° N.	0°	Dr. J. G. Wilson, England
D	Sulphur Mt.	51° N.	58° N.	115° W.	Dr. D. C. Rose, Canada
E	Weissenau	48° N.	49° N.	9° E.	Dr. A. Ehmert, Germany
F	Ottawa	45° N.	57° N.	76° W.	Dr. D. C. Rose, Canada
G	Mt. Norikura	36° N.	26° N.	137° E.	Dr. Y. Miyazaki, Japan
H	Kodaikanal	10° N.	1° N.	77° E.	Dr. V. Sarabhai, India
I	Makerere College	0°	2° S.	32° E.	Dr. D. M. Thomson, Uganda
J	Lae	6° S.	16° S.	147° E.	Dr. A. G. Fenton, Hobart
K	Huancayo	12° S.	1° S.	75° W.	Dr. J. A. Simpson, Chicago
L	Hermanus	34° S.	33° S.	19° E.	Dr. A. M. Vanwijk, Hermanus
M	Mt. Wellington	41° S.	45° S.	147° E.	Dr. A. G. Fenton, Hobart
N	Invercargill	46° S.	52° S.	168° E.	Dr. N. V. Ryder, New Zealand
O	Mawson	67° S.	73° S.	62° E.	Dr. A. G. Fenton, Hobart
1	*H, I, K*	Equator	0–2°		
2	*B, C, D* *E, N, O*	High latitude	49–73°		
3	*D, F, K*	West longitude		75–115°	
4	*C, E, I*	0-Longitude		0– 32°	
5	*J, M, N*	East longitude		147–163°	
6	*A, E, G*	Northern hemisphere	26–76° N.		
7	*L, M, O*	Southern hemisphere	33–73° S.		

In connexion with the Forbush-type decrease in cosmic-ray intensity which occurred on February 11, 1958, we have fortunately a large number of other solar and terrestrial observations which give us a unique set of data for following the event from the time it occurred on the Sun. These have been summarized by Trotter and Roberts[6]. During its second passage on February 9, 1958, a region 58-*B* at 15° S. heliographic latitude, then at the central meridian, suddenly underwent very rapid changes in plage brightness and sunspot growth. The region flared rapidly throughout the day; half a dozen of the flares were Class 1+ or greater. Five of these caused complete short-wave radio fade-outs of considerable duration. In addition, these events were associated with unusual solar radio noise burst activity on 2,800, 470 and 167 Mc./s. The flux density on 167 Mc./s. was very high during February 7–9. An extremely large number of high-speed dark surges were observed on the solar disk, most of them in association with small flares. The mean integrated coronal (5303 A.) intensity was low during the period. Region 58-*B*, which had very intense activity during the second passage in February, persisted with pronounced activity during the third and fourth passages in March and April respectively.

The strongest geomagnetic storm with sudden commencement (s.c.) of the present solar cycle began early on February 11, and almost simultaneously a very spectacular aurora that persisted throughout the night lit up the northern sky as far south as the 35th parallel. It was visible on the following night as well. In Table 1, the important events observed on the Sun and on the Earth are summarized in chronological order.

We have examined the effect in cosmic rays from data of the high counting-rate meson detector at the Massachusetts Institute of Technology and from a grid of neutron monitor stations distributed (1) in two belts corresponding to the equator and the middle geomagnetic latitudes, (2) in three meridional sections corresponding to 75–115° W.,

0–32° E. and 147–168° E., and (3) in the northern and southern hemispheres. In Table 2 are indicated the stations which are included in the grid, particulars of their location and the name of the principal investigator at each station through whose kindness the data have been made available to us. Assuming that the variations can be dependent on the primary energy response, on local time or longitude and on the hemisphere, we have grouped stations so as to study one variable at a time with, so far as possible, an equal contribution in each group due to the other two variables. The stations from which data have been combined for the various analyses are indicated in Table 2.

Fig. 1 shows the percentage deviations in the bi-hourly counting-rates of the neutron monitors during successive bi-hourly periods in U.T. from February 9–12, the deviations in each case being taken with respect to the mean intensity on February 10, which represents a period of 24 hr. immediately preceding the onset of the Forbush decrease early on February 11. It is clear from Fig. 1*A* that the variation is strongly dependent on primary energy. It will be noticed that the meson detector at the middle latitude exhibits a variation which is intermediate between the variation of the neutron monitor intensity at the equator and at the middle latitude stations. The middle latitude stations have a much larger percentage decrease than the stations at the equator. A minimum intensity is reached at 0300 U.T., at 0500 U.T. and at 0700 U.T. at the equator, with the meson detector and at the middle latitude stations respectively. Moreover, about 12 hr. after the initial decrease, at the equator the intensity returns almost to normal before it decreases again ; on the other hand, the recovery occurs only partially at middle latitude stations.

A most interesting aspect of the present event is the increase of intensity at 2100 U.T. on February 10, observed at equatorial stations only, about 4 or 5 hr. before the arrival of the solar plasma at 0120 U.T., indicated by the storm with sudden commencement and a number of other terrestrial effects. The second increase, or the recovery of intensity at 1100 U.T. on February 11, is seen to be much more significant at the equatorial stations and in the meson detector

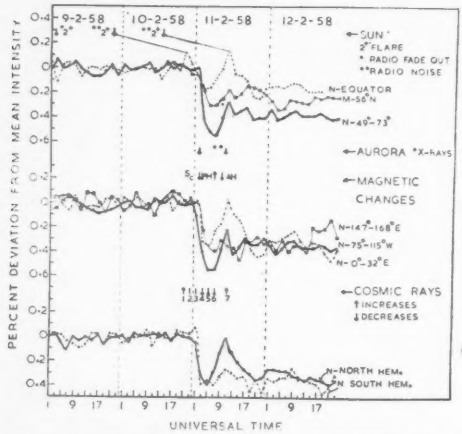

Fig. 1. Cosmic-ray intensity changes and associated solar and terrestrial effects for the cosmic-ray storm of February 11, 1958. Relationships of changes are indicated separately in *A* for low and medium latitudes and primary energy response, in *B* for meridional sections and in *C* for hemispheres

at Cambridge than at the middle latitude stations. It would thus appear that both events, which appear to be increases, are particularly characteristic of the high-energy component of the primary radiation. In contrast, the first minimum of the Forbush event is larger and occurs later for low-energy than for high-energy primaries.

In Fig. 1*B* the variations of intensity at stations in the three meridional belts are compared. It is seen that there are significant differences in the initial decrease, indicating the existence of an anisotropy. The most remarkable feature is the complete absence of the second increase at 1100 U.T. on February 11 at stations in the east meridional section (147° E. to 168° E. longitude), as also at stations in the southern hemisphere for which a comparison with the northern hemisphere is shown in Fig. 1*C*. The second increase of intensity is thus characterized by a strong anisotropy not only parallel to the ecliptic, but also perpendicular to it. This is perhaps the first evidence for an anisotropy of the latter type. In contradiction to the view of Yoshida and Wada, we believe that the second increase is mainly anisotropic and has an energy dependence different from the mainly isotropic Forbush decrease.

The main event observed early on February 11 in cosmic rays, in geomagnetism, in the aurora and in X-rays at high altitudes is undoubtedly related to the major solar outburst from region 58-*B* between 2053 and 2139 U.T. on February 9. We would like to suggest here that solar plasma reached the interaction distance of the geomagnetic field at about 0120 U.T. on February 11, but that for several hours prior to that, there was a cosmic-ray effect which involved an increase of the radiation. During the second increase of cosmic-ray intensity on February 11, we have an increase of cosmic-ray intensity occurring with a strong aurora and change of the horizontal component H of the geomagnetic field. This contrasts with the association of the aurora and the change in magnetic field with the large decrease of cosmic-ray intensity about 10 hr. earlier. From other geophysical evidence it is believed that the main plasma outburst streamed past the Earth in 10–12 hr. and it appears that the second increase of cosmic-ray intensity is related to the departure of the plasma cloud. There was a 2^+ flare with major burst of radio noise at 67° W. heliographic longitude, which occurred at 1325 U.T. on February 10. It is worth while examining whether the second increase is related to the arrival of fresh solar particles from this flare. If, in order to explain the terrestrial influence of a solar event far removed from the central meridian to the west, one postulates the presence of a guiding path of solar magnetic lines of force stretched out to the Earth by earlier streams or an outward solar wind, it would be difficult to explain the 24-hr. delay for solar particles of even a few MeV. energy. We are thus inclined not to associate the second event with the solar outburst on February 10.

We believe that in the two increases and the main decrease observed with the cosmic-ray storm of February 11, 1958, we have essentially three types of modulation process. One is directly associated with the moving plasma, probably related to the magnetic fields in the shock front and gives increases as well as decreases of intensity along with anisotropy. The second gives decreases of intensity and is related to a process which has a sharp onset but a relatively long time constant of recovery. The first is often more effective for high primary energies than low,

but the second is much more effective for low than for high energies.

The large anisotropy parallel to the north–south and east–west directions in the second increase poses an important problem. The different motions of solar particles trapped by the geomagnetic field have been discussed by Gold[7], and before average conditions are established round the globe there is probably a basis for major differences in conditions over the hemisphere and at different meridional sections immediately following the arrival of a new cloud of solar particles. But the time involved is very short compared to the observed effect which shows up over periods of several hours. Moreover, even though changes in the Van Allen radiation belts could perhaps provide an adequate mechanism for the perturbation of the geomagnetic field, and through it alter cosmic-ray intensity, a quantitative evaluation of the effect has not so far been undertaken.

We are grateful to Mr. S. R. Thakore and to the computation section at the Physical Research Laboratory and to Miss Britt at the Massachusetts Institute of Technology for help in analysing data. One of us (V. S.) wishes to express gratitude for the hospitality of the Laboratory for Nuclear Science, Massachusetts Institute of Technology, and for financial assistance from the Department of Atomic Energy of India. The work at the Massachusetts Institute of Technology has been assisted by the joint programme of the Office of Naval Research and the U.S. Atomic Energy Commission, and one of us (R. P.) is supported by a fellowship of the Conselho Nacional de Pesquisas, Brazil, which are gratefully acknowledged. We have had many stimulating discussions with B. Rossi and T. Gold.

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[2] Lockwood, J. A., *Phys. Rev.*, **112**, 1750 (1958).
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MEASUREMENT OF THE EFFECTS OF PISTON MASS AND BURSTING PRESSURE ON THE MOTION OF A PISTON IN A HYPERSONIC GUN TUNNEL

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A HYPERSONIC gun tunnel[1] is essentially a blow-down tunnel with a shock compression heater to generate a high-temperature reservoir of gas. This heater consists of a high-pressure vessel separated from a long barrel by a diaphragm and light piston (see Fig. 1). When the pressure is high enough the diaphragm bursts and drives the piston down the barrel. A shock wave forms ahead of the piston and is reflected from the almost closed nozzle end of the barrel, causing several compressions of the gas within the barrel and raising the temperature. The piston finally comes to rest with the pressures on both

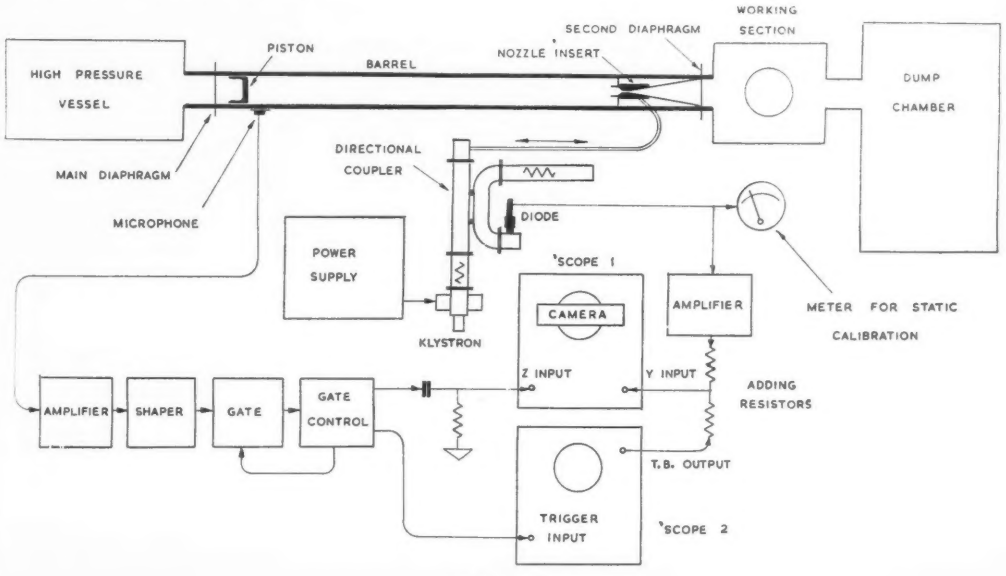

Fig. 1. Layout

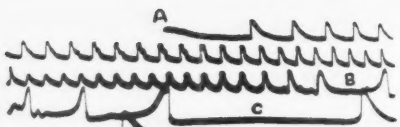

Fig. 2. Typical oscilloscope record

sides equal. Both the mass of the piston and the bursting pressure will affect the performance of the tunnel.

One of the investigations into the operation of the gun tunnel at the Imperial College of Science and Technology, London, was to find how the bursting pressure and piston mass affect the motion of the piston down the barrel. This was done by a microwave technique[2], the barrel being turned into a long resonant cavity by coating the face of the piston with a conducting material, either a silver colloid paint or by gluing and screwing on a dural disk. Microwaves are injected at the nozzle end and resonance occurs with the piston at every half wave-length. By detecting and recording these resonances an analysis of the piston motion is possible.

The generator is a 200-mW., 10-cm. klystron feeding a wave-guide directional coupler. To reduce the tendency for the klystron frequency to follow the changing resonant frequency of the barrel cavity, an attenuator is inserted between the klystron and the directional coupler. The purpose of the directional coupler is to sort out signals according to their direction, so that only the reflected waves coming from the barrel reach the diode. Power is fed from the klystron to the probes mounted in the nozzle insert through a co-axial cable. These probes are arranged not to interfere with the normal working of the tunnel while still exciting a radially symmetric mode (TM_{01}) in the barrel. During a run the probes are protected by a nylon block. The probes are matched to the barrel cavity so that under conditions of resonance there is little power reflected back along the co-axial feeder. However, when the piston moves from the resonant position the subsequent mismatch causes power to be reflected and is detected by the diode on the directional coupler. Hence a resonant point is indicated by a drop in the voltage output from the diode. The drop in voltage is recorded by an oscilloscope and camera. About fifty resonant points have to be measured and, as one sweep of the cathode ray tube has insufficient resolution, a raster type of display was used with a second oscilloscope providing the vertical time-base. Oscilloscope 1 was arranged to sweep continuously but with the beam blanked off, and oscilloscope 2 brightened up the first beam when triggered from a bursting signal picked up by a microphone. The time-base from the second oscilloscope was then added to the diode output to displace consecutive lines on the recording tube, producing a raster display. One trouble encountered was that the time-base voltage biased off the diode and reduced the signal to nothing. This was cured, with the attendant advantages of a larger signal, by inserting a small self-contained transistor isolating amplifier between the diode and the adding resistors (see Fig. 1). The amplifier has a voltage gain of 17, a frequency response of 15 c./s. to 500 kc./s. ± 3 db. with a high input and low output impedance. Two *OC* 45 transistors are used. A similar amplifier was also used as a microphone amplifier. Because of the resulting 'ring' and general noise of a burst, several traces were superimposed for each run as the second oscilloscope triggered more than once. To ensure that only one trace was obtained a trigger and gate system was adopted. This amplifies and shapes incoming signals from the microphone and passes them to a gate. A pulse coming from this gate closes the gate

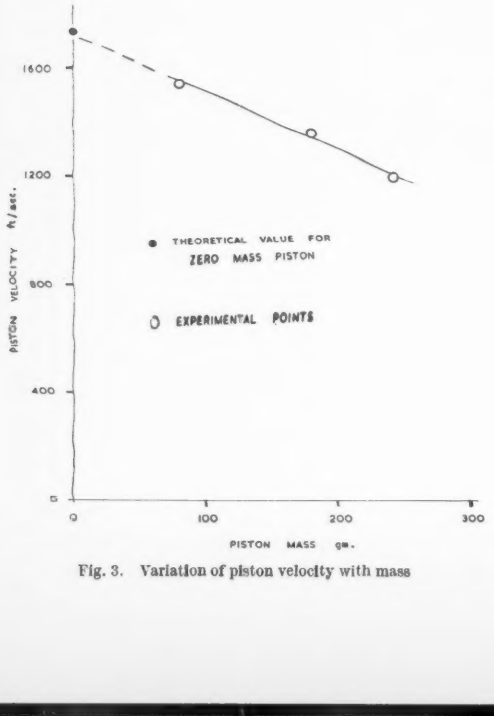

Fig. 3. Variation of piston velocity with mass

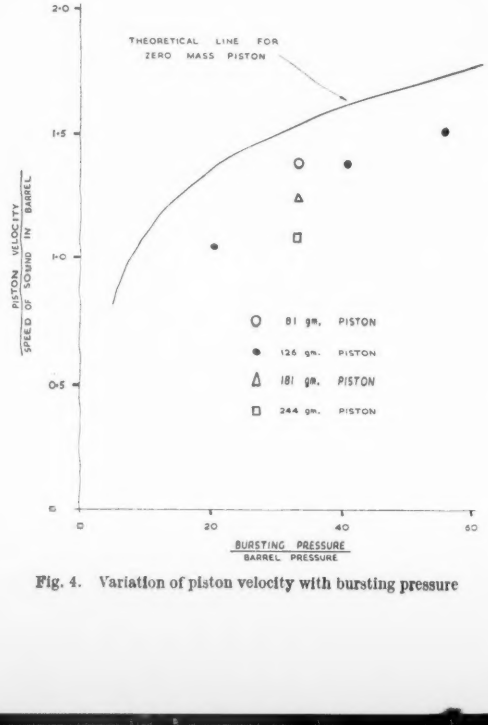

Fig. 4. Variation of piston velocity with bursting pressure

and triggers the second oscilloscope. Hence only one trace is possible before the system is reset by opening the gate manually.

A typical record is shown in Fig. 2. Starting at A, which is arranged to be on a resonant point for reference, the distance between pips becomes less, indicating acceleration of the piston, until the third line down where after a rapid deceleration the wave-shape is seen to reverse, at B, indicating that the piston has reversed and is now going the other way down the tube. On the fourth line at C another reversal is evident. The resonant points are indicated by positive pips because the amplifier reverses the sign of the signal.

These film records were analysed to give a plot of piston position (x) against time (t). From such plots the maximum piston velocity is taken. Figs. 3 and 4 summarize these results. Initial acceleration was also measured from the x–t plots assuming the acceleration to be constant over the first few stations, the results being :

Bursting pressure (lb./sq. in.)	Acceleration
300	4,000g
485	5,400g
600	7,200g
825	15,000g

I am indebted to Mr. J. L. Stollery for his direction and help in the foregoing work, and to Mr. P. D. Church for his analysis of the traces.

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A STRUCTURAL MODEL FOR MONATOMIC LIQUIDS INCLUDING METALLIC LIQUIDS

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ALTHOUGH the melting point (T_m) is generally determined thermodynamically, it is interesting that Lindemann's geometrical law[1] is effective in determining the melting condition of solids. So we have studied the structure of liquids at their melting points in the hope of finding some similar law.

So far, radial distribution curves for 18 monatomic liquids near their melting points have been obtained by X-ray or neutron diffraction measurements, and they give co-ordination numbers (Z) and the distance (r_1) of the nearest neighbour. However, the values of Z so far obtained are less reliable than those of r_1; it is probable that in some cases we obtained low values. Therefore, we calculated Z from r_1 and the bulk density (measured) assuming a quasi face-centred cubic lattice. The calculated values $Z_{cal.}$ were between 10 and 11·5 (mean value 10·85, about 90 per cent of 12. Table 1).

Table 1. Data for Radial Distribution Curves near T_m

	r_1 (A.)	$Z_{ob.}$	$Z_{cal.}$	$A_{ob.}$ (A.)	$A_{cal.}$ (A.)*	r (3)
He (*a*)	3·85	10·5	10·5	2·85	3·36	—
Ar (*b*)	3·86	8·0	10·5	3·40	3·37	—
Xe (*c*)	4·43	9·4	10·4	3·84	3·87	—
Li (*d*)	3·24	9·8	*12·7*	*2·60*	2·83	2·74
Na (*e*)	3·83	9·5	11·2	3·21	3·34	3·43
K (*f*)	4·64	8·0	10·8	3·95	4·05	*4·36*
Au (*g*)	2·86	11·0	10·4	2·42	2·49	2·52
Zn (*d*)	2·94	10·8	*13·7*	*2·33*	2·57	*2·40*
Cd (*d*)	3·06	8·3	10·4	2·60	2·67	2·72
Hg (*h*)	3·10	8·3	10·3	2·68	2·70	2·78
Al (*d*)	2·96	10·6	11·6	2·50	2·58	2·50
Ga (*g*)	2·77	11·0	9·5	2·43	2·42	2·45
In (*d*)	3·30	8·5	11·3	2·86	2·88	2·96
Tl (*g*)	3·30	8·0	10·1	2·85	2·88	3·06
Ge (*g*)	2·70	8·0	—	2·26	2·36	2·37
Sn (*i*)	3·23	9·1	10·7	2·79	2·82	2·77
Pb (*j*)	3·40	9·4	10·3	2·93	2·96	3·02
Bi (*j*)	3·40	8·0	9·6	2·90	2·97	3·04

(*a*) Gordon, Shaw and Daunt, J. *Phys. Chem. Solids*, **5**, 117 (1958). (*b*) Eisenstein and Gingrich, *Phys. Rev.*, **62**, 261 (1942). (*c*) Campbell and Hildebrand, *J. Chem. Phys.*, **11**, 334 (1943). (*d*) Gamertsfelder, *J. Chem. Phys.*, **9**, 450 (1941). (*e*) Trimble and Gingrich, *Phys. Rev.*, **53**, 278 (1938). (*f*) Thomas and Gingrich, *J. Chem. Phys.*, **6**, 411 (1938) and Gingrich and Wall, *Phys. Rev.*, **56**, 336 (1939). (*g*) Hendus, *Z. Naturforsch.*, **2a**, 505 (1947). (*h*) Vineyard, *J. Chem. Phys.*, **22**, 1665 (1954). (*i*) Takeuchi and Furukawa, Meeting of Japan Inst. Metals (April, 1958). (*j*) Sharrah and Smith, *J. Chem. Phys.*, **21**, 228 (1953). *$A_{cal.} = r_1/(1·5)^{1/2}$.

The shape of the curves was analysed by Wall's theory[2] using the spherical free volume of radius σ. According to this theory, $(r_1 - \sigma) = A$ corresponds to the position on the shorter side-branch at a height of 34·4 per cent of the maximum value in the first peak of the curve $4\pi r\rho(r)$, where $\rho(r)$ is the atomic number density at a distance r from any atom. Hence we measured $A_{ob.}$ from radial distribution curves, and obtained the empirical formula : $r_1/A_{ob.} = (1·5)^{1/3}$ (Table 1).

Assuming a molecule to be a sphere (nearly rigid)[2] of diameter A at T_m, the following model can be postulated : Let V_0 be the volume at closest packing of spherical molecules of diameter A, then the volume of liquid at T_m is $1·5\ V_0$ for quasi face-centred cubic lattice. But about 10 per cent of the sites in this quasi-lattice are empty, and these spaces are distributed through all interstices, explaining the second peak at $1·9r_1$ of the distribution curves. Thus the total volume is about $1·65\ V_0$.

Using this model of a reduced form independent of materials, several properties of liquids can be explained as follows. Self-diffusion and viscous flow in liquids are easily explained. The self-diffusion coefficient of a liquid at its melting point may be expressed[3,4] as: $D_m = \gamma\, r_1^2\, \nu_m \exp(\Delta S_D/R) \exp(-H_D/RT_m)$, where γr_1^2 is the mean square of jump distances, ν_m the frequency of the liquid at T_m and ΔS_D and H_D the activation entropy and energy of diffusion respectively. Now γ, ΔS_D, H_D/RT_m and $D_m/(r_1^2\, \nu_m)$ must all be constants independent of the material. If ν_m is calculable from Lindemann's formula, $\nu_m = 2·8 \times 10^{12}\, T_m^{1/2}\, M^{-1/2}\, V_m^{-1/3}$, where M is the molecular weight and V_m the molar volume, $D_m/(r^2{}_1\, \nu_m)$ is obtainable from experimental values of D_m, and is nearly constant (Table 2).

The experimental values of H_D/T_m and $H\eta/T_m$ are also nearly independent of the material, where $H\eta$ is an activation energy of viscosity (Table 2). Differences between the liquid metals and other material may be explained by the expansion co-

Table 2. Self-diffusion and Viscosity Data (c.g.s.)

	$(D_m/r_1^2\nu_m)\times10^2$	H_D/RT_m	$H\eta/RT_m$	$\eta_{m,ob.}\times10^3$	$\eta_{mcal.}\times10^3$
Na	0·72 (*a*)	3·28 (*a*)	2·2 (*d*)	0·71 (*d*)	0·59
Hg	0·83 (*a*)	2·15 (*a*)	1·41 (*d*)	2·1 (*d*)	2·07
In	0·73 (*a*)	2·83 (*a*)	1·86 (*c*)	1·94 (*c*)	1·97
Ga	0·79 (*a*)	1·85 (*a*)	1·61 (*d*)	2·14 (*d*)	1·63
Sn	1·32 (*b*)	3·96 (*b*)	1·78 (*c*)	1·95 (*c*)	2·19
Ag	0·74 (*c*)	3·30 (*c*)	1·91 (*f*)	3·88 (*f*)	4·06
Ar	0·98 (*a*)		3·12 (*g*)	0·28 (*g*)	0·11
N_2			3·69 (*g*)	0·31 (*g*)	0·23
CO			3·50 (*g*)	0·32 (*g*)	0·23
CH_4			4·14 (*h*)	0·23 (*h*)	0·20
C_6H_6	0·41 (*k*)	4·99 (*k*)	4·31 (*i*)	0·83 (*i*)	0·90
O_2			3·70 (*g*)	0·81 (*g*)	0·27
CCl_4	1·87 (*a*)	6·00 (*a*)	4·74 (*j*)	2·0 (*j*)	0·55

(*a*) See ref. 12 concerning the experimental values of D_m. (*b*) Careri and Paoletti, *Nuovo Cim.*, 11, No. 3, 574 (1955). (*c*) Yang, Kado and Derge, *Trans. Met. Soc. A.I.M.E.*, **212**, 628 (1958). (*d*) "Liquid Metal Handbook" (1952). (*e*) Culpin, *Proc. Phys. Soc.*, B, **70**, 1069 (1957). (*f*) Gebhardt and Wörwag, *Z. Metk.*, **42**, 358 (1951). (*g*) Rudenko and Schubnikow, *Phys. Z. Sowjet.*, **6**, 470 (1934). (*h*) *ibid.*, **8**, 179 (1935). (*i*) Grunberg and Nissan, *Trans. Farad. Soc.*, **45**, 125 (1949). (*j*) Thorpe and Rodgen, *Phil. Trans. Roy. Soc.*, A, **185**, 397 (1894). (*k*) See ref. 6.

efficients the ratio of which is of the order of 10^{-2}. In fact, under the condition of constant volume H_D/RT_m becomes 2·14 for CCl_4[5], and 1·26 for C_6H_6[6], from isobaric experiments, and becomes 2·07 for Hg[4], 1·63 for Ga[4], 1·65 for CCl_4[5] and 1·40 for C_6H_6[6] by computation from isothermal experiments using[6] their expansion coefficients and compressibilities.

So putting $H_D/RT_m \equiv 1{\cdot}6$ at constant volume, we obtain $\Delta S_D/R = -3{\cdot}1 \pm 0{\cdot}3$ independent of the materials from their values $D_m/(r_1^2\,\nu_m)$ of Table 2. $\Delta S_D < 0$ can easily be explained by a more regulated activated-complex configuration composed of planar 4 or 5 atoms in the closest contact than the ordinary irregular configurations. Considering the above, it is of interest that A nearly coincides with the metallic bond-lengths of co-ordination number 3 calculated by Pauling's theory[7] (r (3) in Table 1).

Combining these facts with the Sutherland-Einstein formula[8], $D_\eta = kT/(2\pi A)$, we can easily deduce Andrade's formula[9] $\eta_m = 5{\cdot}7 \times 10^{-4}\, M^{1/2}\, T_m^{1/2}\, V_m^{-2/3}$ putting $D_m/(r^2_1\,\nu_m) = 0{\cdot}0067$. The calculated viscosity coefficients at T_m, $\eta_{m,cal.}$, are in good agreement with the experimental values $\eta_{m,ob.}$ (Table 2).

Table 3. Entropy of Liquids at T_m (cal./deg./mole)

	Calculated	Observed		Calculated	Observed
Ar	12·458	12·594 (*a*)	*K*	18·154	17·816 (*c*)
Xe	18·704	19·04 (*b*)	Au	23·613	23·32 (*d*)
Li	11·170	11·001 (*c*)	Al	16·820	17·00 (*d*)
Na	15·130	15·507 (*c*)	Pb	22·412	22·28 (*d*)

(*a*) Clusius and Frank, *Z. Elektrochem.*, **49**, 308 (1943). (*b*) Clusius and Roccobini, *Z. phys. Chem.*, **38B**, 81 (1937). (*c*) Evans *et al.*, *J. Res. Nat. Bur. Stand.*, **55**, 83 (1955). (*d*) See Borelius, "Solid State Phys.", **6**, 55 (1958).

Moreover, using Wall-Harasima's free volume theory[2,10], and adding the entropy of random arrangement of vacancies as a cruder treatment of this model, the entropies of the liquids at their melting points were calculated; the values agreed with the observed ones (Table 3).

The above discussion shows that the metallic liquid is not a special liquid. Thus we need not consider the ionic unit[4,11,12] in the transport phenomena.

The model will become more complete when combined with the recent work of Bernal[13], who explained the essential difference between regular and irregular close-packing arrangements having a volume difference of about 10 per cent, which agrees with our model.

A detailed description including further applications will be published elsewhere.

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POLYNUCLEAR COMPLEXES OF MOLYBDENUM(II)

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ALTHOUGH molybdenum(II) chloride and its co-ordination complexes have been known for a century, they have received but little attention. As a result of the virtual absence of physical studies on these compounds, not only was nothing known of their constitution until a few years ago, but incorrect molecular formulæ were even adopted. The relatively recent X-ray diffraction studies by Brosset[1-3] have done much to improve the position, though the bonding present in molybdenum(II) chloride derivatives has never been seriously discussed and many simple physical measurements remain to be done and correlated with the proposed structures. A re-investigation of these compounds is now particularly important as they appear to possess a stereochemistry quite different from that indicated for d^4 metal complexes by ligand field theory. Therefore molybdenum(II) chloride and its derivatives are being re-examined and it is now possible to summarize some new experimental results and propose a bonding scheme for these compounds. This bonding scheme is able to explain why only certain types of ligands form molybdenum(II) chloride complexes.

The crystalline compounds studied by Brosset were formulated on the basis of his structure determinations as $[(Mo_6Cl_8)\{(OH)_4(H_2O)_2\}].12H_2O$ [1] and $[(Mo_6Cl_8)\{Cl_4(H_2O)_2\}].6H_2O$ [2]. The structure of the $(Mo_6Cl_8)^{4+}$ group, fully named octa-μ_3-chlorohexamolybdenum(II) and henceforth referred to as the chloromolybdenum(II) group, is given in Fig. 1. It is convenient for most purposes to regard the group

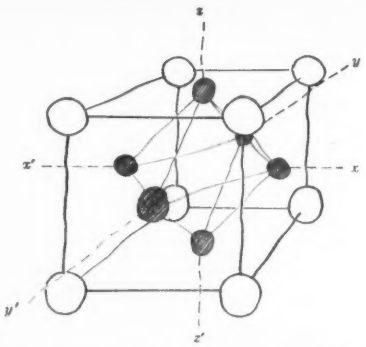

Fig. 1. The chloromolybdenum(II) group. White circles, Cl; shaded circles, Mo

as a regular face-centred cube and it is presented in this manner in the figure. Though the octahedron described by the molybdenum atoms is almost regular, the cube described by the chlorine atoms is somewhat distorted and it is possible that the deviation from strict equivalence of these chlorine atoms may be of some chemical significance. Brosset has emphasized that six groups, given in braces in the formulæ above, appear closely associated with the chloromolybdenum(II) unit. Fig. 1 shows that each molybdenum atom is surrounded by a square planar configuration of chlorine atoms, and can accept one ligand normal to the cube face to achieve co-ordinative saturation. The effective co-ordination number of each molybdenum atom, taking into account the four molybdenum close neighbours, would be nine, and it is therefore improbable that more than one ligand could donate to each molybdenum atom. Thus six ligands may be accepted by the chloromolybdenum(II) group along the axes xx', yy' and zz'. Whereas the identity of the central (Mo_6Cl_8) unit is seldom affected, the six ligands are freely variable. There is possible a novel range of octahedral complexes, their general character being of the type familiar to the inorganic chemist, but possessing a polyatomic nucleus.

The chloromolybdenum(II) group is stable over a wide range of conditions. In the form $(Mo_6Cl_8)Cl_4$, that is, $MoCl_2$, it is not affected by boiling aqua regia or concentrated sulphuric acid heated to fuming, other than the displacement of the chlorine ligands. It is stable to more than 800° C. *in vacuo*, with probable disproportionation to metal and higher halides above this temperature. It is not oxidized by air below 300° C., but above this temperature a dark product is formed. However, it is much more sensitive to aqueous alkali giving molybdenum(V) hydroxide and hydrogen. At room temperature and *p*H 12, a chloromolybdenum(II) solution shows some signs of decomposition in about 10 hr., though at higher *p*H and temperature decomposition can be complete in a few minutes. It is noteworthy that the reagents most disruptive for the (Mo_6Cl_8) group are the strong electronegative complexing agents, namely, OH^-, F^- and NCS^-, though attack by these is rapid only at high temperatures. It is therefore possible that the decomposition of the chloromolybdenum(II) group under these conditions proceeds by the formation of an unstable substituted chloromolybdenum(II) nucleus. As this group retains its identity under most conditions, it is proposed to designate it as '*M*' in formulæ.

The present work strongly supports the view that the chloromolybdenum(II) group invariably possesses six groups bound to it. The following are typical examples of the new compounds isolated: $[MCl_4.(C_5H_5N)_2]$; $[MCl_4.((C_2H_5)_3N)_2]$; and $(H_3O)_2[MI_6].6H_2O$. Compounds with compositions corresponding to the following have also been reported[4]: $[MCl_4.(H_2O)_2]$; $[MCl_4.(C_2H_5OH)_2]$; $K_2[MCl_6].6H_2O$; $[MBr_4.(H_3O)_2]$; and $(H_3O)_2[MBr_6].6H_2O$. The parent acid of the hexachloro-chloromolybdenum(II) series has been known in the crystalline form as $(H_3O)_2[MCl_6].6H_2O$ for some time[5]. It is the most soluble hexachloro-complex, the salts of this acid being sparingly soluble or completely insoluble. Typical salts include those of the alkali metals, ammonium and pyridinium[4]. The insoluble salts derived from large organic cations, for example, tetraethylammonium and triphenylphosphonium, have been prepared in the present work. The salts of the hexabromo- and hexaiodo-acids are less well known but are undoubtedly similar to those of the hexachloro-acid. The hexahalogeno-acids and their salts are rapidly hydrolysed in aqueous solution and an excess of hydrohalogenic acid must be maintained for stable solution. Solvolysis of these compounds is not observed in ethanol and solubilities often prove to be much higher in this solvent.

In addition to MCl_4, it has now been possible to prepare MBr_4, MI_4 and $M(OH)_4$ by heating the appropriate hexahalogeno-acid or hydrated chloromolybdenum(II) hydroxide *in vacuo* at 250° C. As there appears to be only four ligands per chloromolybdenum(II) nucleus it is reasonable to suppose that these compounds are polymeric using some ligands for bridging between chloromolybdenum(II) groups and thus satisfying the proposed octohedral requirements. However, it is interesting to note that Brosset has shown the hydrates of MCl_4 and $M(OH)_4$ to consist of discrete octahedral complexes. In passing, it may be mentioned that the four simple chloromolybdenum(II) compounds referred to above are found to be hygroscopic and undergo a lightening of colour on gaining water. The materials may be obtained anhydrous again by reheating *in vacuo*.

Complexes of the type $[M(OH)_6]^{2-}$, $[M(H_2O)_6]^{4+}$ and $[M(NCS)_6]^{2-}$ are of interest but attempts to prepare solid compounds of the first two have failed so far. Such compounds undoubtedly exist in solution as chloromolybdenum(II) hydroxide is soluble in 2 *N* acid and 0·01 *N* alkali. It has been possible to confirm the existence of $[M(OH)_6]^{2-}$ in solution by the *p*H titration involving the precipitation of the hydroxide by standard acid from a standard alkaline solution. Fig. 2 shows a typical titration curve in which two equivalents of nitric acid precipitate one of $M(OH)_4$. Thus chloromolybdenum(II) hydroxide dissolves in alkali to give the complex $[M(OH)_6]^{2-}$. The addition of $[MCl_6]^{2-}$ to a concentrated potassium thiocyanate solution gives a crystalline precipitate possessing a chloride and thiocyanate content reasonable for $K_2[M(NCS)_6].6H_2O$. However, the compound is soluble in water, precipitating only in excess thiocyanate, and attempts to free the precipitate from the contaminating potassium thiocyanate have not proved successful so far.

The ideas expressed above might be criticized on the grounds that the chloromolybdenum(II) nucleus or its proposed co-ordination complexes are, in fact, ionic assemblages rather than covalent compounds. Though this is inconsistent with the chemical data already given, there is good evidence to settle this

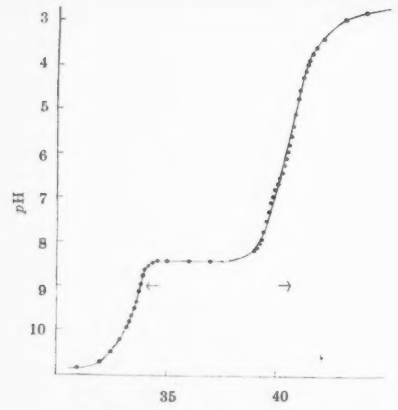

Fig. 2. The precipitation of $3{\cdot}20 \times 10^{-4}$ moles of $M(OH)_4aH_2O$ occurred in the region between the arrows

point. Brosset has established that the hexachloro-acid exists as $[MCl_6]^{2-}$ in ethanolic solution by an X-ray diffraction technique[3]. Molecular weight determination of molybdenum(II) chloride in boiling ethanol has been found to give just over half the required value for MCl_4 [6]. Under these conditions, it is very probable that some alcoholysis occurred, but ionization to a degree suggested by $M^{4+}.(Cl^-)_4$ as the true constitution of molybdenum(II) chloride is ruled out. Thus, in a moderately strong solvating solvent, like ethanol, the *M*—Cl bonds retain their identity. A number of chlorine-36 exchange studies to determine the lability of the chlorine atoms in the $[MCl_6]^{2-}$ ion have been carried out in the present investigation. The first experiments were carried out in 5·65 *N* hydrochloric acid to avoid hydrolysis. The hexachloro-acid was allowed to exchange with labelled hydrochloric acid, and the complex separated from solution either by freezing out at 0° C. as the crystalline acid or precipitated as the triphenylphosphonium salt. The first separation method gave a very pure material, the second gave a rapid quantitative precipitation. It was found that the exchange fraction corresponded closely to the exchange of only six out of the fourteen chlorine atoms in $[MCl_6]^{2-}$ for exchange times of 2–800 min. at 25° C. For times of exchange less than 2 min., less than six out of fourteen chlorine atoms exchanged, but the data are inaccurate. It is reasonable to conclude that all fourteen chlorine atoms in $[MCl_6]^{2-}$ are covalently bound, but the ligand chlorine atoms are much more labile than the nuclear chlorine atoms in 5·65 *N* hydrochloric acid. The inertness of the eight nuclear chlorine atoms is striking, for on refluxing the hexachloro-acid for 1½ hr. in hydrochloric acid, only six out of fourteen had exchanged.

At this point it is logical to consider whether chloromolybdenum(II) should have a chemistry analogous to some mononuclear cation, or whether the resemblance is superficial. It is true that if the molybdenum(II) atoms were held together by a cage of shared chlorine atoms, it may well be that the compounds resembling octahedral complexes could be isolated. However, these would have the properties expected of divalent molybdenum, frequently displaying paramagnetism, ligand field spectra and a strong tendency to oxidation. This is in striking contrast to what is observed, for chloromolybdenum(II) complexes are very stable to oxidation and are all diamagnetic. Solution absorption spectra give no indication of ligand field spectra, only one or more bands at about 300–350 mμ, $\varepsilon \sim 3 \times 10^3$, which account for the deep yellow colour of the compounds. It is possible, however, that these charge transfer bands may be superimposed on some very weak *d*–*d* transition bands. Fig. 3 gives the absorption curves assigned to the species $[MCl_6]^{2-}$ and $[MBr_6]^{2-}$ in 5 *N* hydrochloric and hydrobromic acid, respectively, and $[M(OH)_6]^{2-}$ in 0·01 *N* alkali. It is important to bear in mind that the properties suggested above for molybdenum(II) are hypothetical as no paramagnetic molybdenum(II) compounds are known and complexes of the type $[Mo(II)X_6]$, from which $[MX_6]$ might be considered to be derived, do not exist. Furthermore, mononuclear molybdenum(II) compounds are very rare (see below). If molybdenum(II) is spin-paired in chloromolybdenum(II), accounting for its diamagnetism, combination of this group with carbon monoxide-like ligands would be expected, and again this is not found. There is no evidence that MCl_4 is affected by carbon monoxide at 40 atm. and 110° C. or by triphenylphosphine at 200° C. It is significant that among the few mononuclear molybdenum(II) complexes known, $[Mo(II)(diarsine)(CO)_3I_2]$, $[Mo(II)(diarsine)_2(CO)_2I^+].I^-$ and similar compounds have recently been prepared by Nyholm and co-workers by halogenation of tetracarbonyl-(*o* - phenylene - *bis*(dimethylarsine))molybdenum(0)[7]. These compounds are diamagnetic, contain carbon monoxide-like ligands and possess ligand field spectra and co-ordination number seven. Therefore, the chloromolybdenum(II) group behaves quite differently from that reasonably expected on molybdenum(II), and moreover, differently from any transition metal other than one with a d^6 configuration. It is then justifiable to regard the chloromolybdenum(II) group as a particular and distinct chemical entity and not as an assemblage of atoms in a rather special geometry.

The non-appearance of typical transition metal properties is not difficult to explain. The intermolyb-

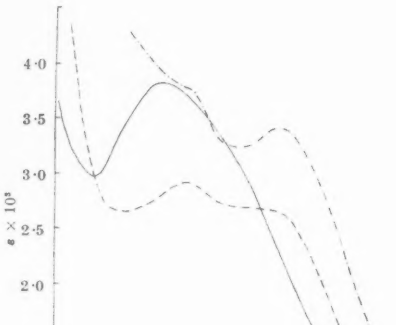

Fig. 3. —·—, $[MBr_6]^{2-}$; ——, $[M(OH)_6]^{2-}$; – – –, $[MCl_6]^{2-}$

denum distance is 2·63 A. to be compared with 2·73 A. for molybdenum metal, and therefore Mo–Mo bonds must exist along all sides of the Mo_6 octahedron. The highly symmetrical arrangement of the Mo_6 group allows convenient consideration of the intermolybdenum bonds as delocalized and best represented by molecular orbitals. For the purposes of discussion the $[MCl_6]^{2-}$ complex will be considered. Each molybdenum atom is surrounded by a tetragonal pyramid of chlorine atoms, bonded probably by $d_{(x^2-y^2)}sp^3$ hybrid orbitals. Of the remaining four d-orbitals, the d_{xz} and d_{yz} possess lobes directed exactly along the intermolybdenum axes and it is possible for each of four co-planar molybdenum atoms to combine one of these orbitals to give a molecular orbital system. There are three such sets of co-planar atoms, each forming such orbitals. The contribution of two d-electrons per molybdenum atom to forming such intermetallic bonds gives molybdenum a valency higher than the oxidation number two. The disposition of the two remaining electrons in the d_{xy} and d_{z^2} orbitals must be consistent with the observed properties of chloromolybdenum(II) compounds. Pairing the electrons in one of the two remaining orbitals may account for diamagnetism, but possibly not the other properties. Furthermore, the proximity of the molybdenum atoms to each other also suggests that there would be some repulsive interaction between non-bonding pairs in either the d_{xy} or d_{z^2} orbitals. It is concluded that the two remaining electrons occupy the orbitals singly, and that these contribute a little more to the intermolybdenum binding by coupling their spins.

Therefore, chloromolybdenum(II) can best be regarded as a compound of molybdenum(VI) employing all nine orbitals. This is quite consistent with crystallographic evidence, for Brosset reports that each molybdenum atom possesses nine neighbours all closer than 2·7 A. The hexavalency of molybdenum explains the absence of observable d–d transitions in spectra and the inability to form complexes with π-bonding ligands. The apparent conflict of the stereochemistry of chloromolybdenum(II) with ligand field theory is removed. Though the theory suggests a number of favoured arrangements for d^4 complexes, it seems unable to account for the arrangement in chloromolybdenum(II). This difficulty disappears when the compounds are recognized as d^0 complexes.

In conclusion, the following points are re-emphasized. Molybdenum(II) chloride complexes contain the chloromolybdenum(II) group, $(Mo_6Cl_8)^{4+}$, which functions as a nucleus for octahedral complexes of the form $[MX_6]$. The chloromolybdenum(II) group exhibits intermetallic bonding, and though the oxidation number of molybdenum is two, the valency is effectively six. Consistent with the d^0 character of the molybdenum is co-ordination number nine. Furthermore, this d^0 character explains the diamagnetism, absence of both ligand field spectra and complexes with π-bonding ligands of chloromolybdenum(II) compounds.

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MASSIVE INCORPORATION OF 5-FLUOROURACIL INTO A BACTERIAL RIBONUCLEIC ACID

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IN the course of studies on the correlation of the mechanisms controlling the production of cellular high-polymers, we have investigated the effects of 5-fluorouracil on the synthesis of protein and nucleic acid in several strains of *Escherichia coli*. We have reported elsewhere (ref. 1, and unpublished work by the same authors) on the ability of the fluoro compound to substitute, in part, for uracil in a mutant requiring this pyrimidine. In the absence of uracil, the addition of 5-fluorouracil resulted in the doubling of the protein content; a slight increase in ribonucleic acid, but none in deoxyribonucleic acid, took place. In *E. coli*, strain *B*, as well as in the uracil auxotroph supplemented with uracil, the fluoro pyrimidine inhibited the synthesis of deoxyribonucleic acid completely, but permitted the formation of both protein and ribonucleic acid.

The formation, in the presence of 5-fluorouracil, of several constitutive or inducible enzymes was also examined. The activity of two of the enzymes, catalase and succinate dehydrogenase, increased; the induction of β-galactosidase by lactose was, on the other hand, almost entirely blocked in the presence of 5-fluorouracil. The fluoropyrimidine prevented, moreover, any further rise of the β-galactosidase activity in cells that had previously been treated with the inducer or in a strain of *E. coli* in which this enzyme is constitutive. Other observations, soon to be published in collaboration with Drs. F. Goodman and J. J. Saukkonen, showed that 5-fluorouracil prevented the multiplication of *T2r* bacteriophage and the intracellular synthesis of the phage nucleic acid in *E. coli*, strain *B*, whereas it had less influence on the growth of *T3*.

These findings, as well as the recent reports on the incorporation of 5-fluorouracil into the ribonucleic acids of animal tissues[2,3] and of tobacco mosaic virus[4], made it of interest to ascertain whether also in *E. coli* the biological effects of the fluoro compound are accompanied, or caused, by its

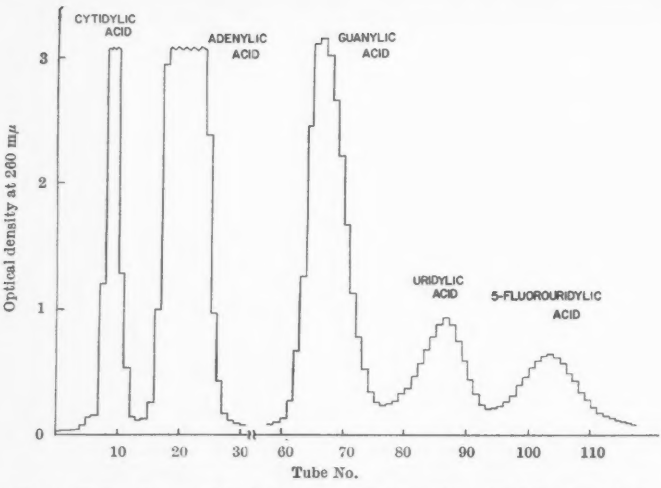

Fig. 1. Elution diagram of the hydrolysate of the ribonucleic acid of *E. coli*, strain *B*, formed in the presence of 5-fluorouracil. 'Dowex-2-formate' (8 times), 200–400 mesh; 23 cm. × 0·9 cm. diameter; gradient elution (mixing chamber: 500 ml. water; reservoir: 2·5 *N* formic acid); 7 ml. fractions

introduction into the ribonucleic acid. The results presented here show that a considerable amount of this uracil analogue does indeed find its way into the ribonucleic acid of the organism, where it may replace nearly one-half of the normally present uracil.

To cultures (37°, glucose-salts medium, early logarithmic phase) of *E. coli*, strain *B*, or of the uracil-deficient mutant 63–86, supplemented with uracil (20 μgm./ml.), 5-fluorouracil (50 μgm./ml.) was added and the cell suspensions were kept at 37° for varying periods. If the action of the inhibitor on the uracil auxotroph was to be tested in the absence of uracil, the cells were harvested and starved before the addition of the fluoropyrimidine[1]. At the conclusion of the treatment, the organisms were collected and prepared for analysis (removal of mononucleotides, lipids, etc.) by procedures described previously[5]. The hydrolysis of the ribonucleic acid was carried out with 0·3 *N* alkali (30°, 18 hr.) with the use of either sodium hydroxide, in which case the hydrolysate was acidified with hydrochloric acid in order to precipitate protein and deoxyribonucleic acid, or of potassium hydroxide; in the latter case perchloric acid was employed, care being taken to avoid losses through the co-precipitation of nucleotides during the removal of potassium perchlorate in the cold. The ribonucleotide composition was determined by electrophoresis on filter paper or by ion-exchange chromatography.

A typical elution pattern (Fig. 1) shows five components, with the new nucleotide leaving the column after uridylic acid, as expected for a mixture of the 2′- and 3′-phosphates of 5-fluorouridine[3]. The combined eluates of this component collected in several runs were again subjected to chromatography; the experimental conditions were as in Fig. 1 except that *N* ammonium formate was used for gradient elution. A single sharp peak was observed and the nucleotide identified as 5-fluorouridylic acid. The hydrolysis of the compound with 72 per cent perchloric acid (1 hr., 100°) liberated 5-fluorouracil, which was identified by chromatography and spectrophotometry; the molar ratio of 5-fluorouracil to phosphorus was found to be 1 : 1·17. The treatment of the nucleotide with prostate phosphatase resulted in the liberation of 5-fluorouridine, which was identified by the comparison of its spectral and electrophoretic (0·1 *M* borate buffer of *p*H 9·2 [6]) properties with those of the authentic nucleoside. The ratio of 5-fluorouridine to phosphate released by the enzymic hydrolysis was 1 : 1·1. That the nucleotide consisted of a mixture of the 2′- and 3′-phosphates of 5-fluorouridine and had, therefore, formed part of a polynucleotide, was shown by its behaviour towards 3′- and 5′-nucleotidases. It was not attacked by the 5′-nucleotidase of Russell's viper venom, whereas 3′-nucleotidase[7] hydrolysed about 60 per cent of the nucleotide to fluorouridine within 20 hr. 5-Fluorouridylic acid has its absorption maximum at 268 mμ in 0·01 *N* hydrochloric acid with a molar extinction coefficient of 9,400; the absorbance ratios in the same solvent are A_{250}/A_{260}, 0·64; A_{280}/A_{260}, 0·82; A_{230}/A_{260}, 0·32.

The nucleotide composition of the ribonucleic acid synthesized, both in the presence and the absence of 5-fluorouracil, by the two *E. coli* strains under investigation is shown in Table 1. It will be seen that one-quarter to nearly one-half of the nucleic acid uracil can be replaced by the fluoropyrimidine. This takes place without an essential disturbance of the equality of the molar sums of 6-amino and of 6-keto nucleotides[10]. The large quantities of 5-fluorouracil built into the ribonucleic acid of strain *B* offer a convenient method for the preparation of the 2′-, 3′-, and 5′-phosphates of 5-fluorouridine. It is noteworthy that 5-fluorouridylic acid was also found in the ribonucleic acid of the uracil-requiring mutant incubated with the fluoropyrimidine in the absence of uracil, although the amount of nucleic acid synthesized under these conditions is slight (ref. 1, and unpublished work by the same authors). Owing to the relatively small number of analyses it is not yet clear what importance should be attached to the fluctuations in nucleotide proportions recorded in Table 1; nor is it yet known whether 5-fluorouracil is incorporated preferentially into any particular fraction of the total ribonucleic acid or into certain positions on the polymer chain.

In contrast to the known metabolic fate of uracil, we have found no indication that 5-fluorouracil gives rise also to a 5-fluorocytidylic acid component of the nucleic acid. Preliminary evidence would, in fact, seem to speak against the presence of the latter fluoro nucleotide, at least in amounts comparable to those of 5-fluorouridylic acid. Neither elution peak nor chromatographic zone corresponding to 5-fluorocytidylic acid was seen; the spectra of the separated nucleotides showed no evidence of such a contaminant. Moreover, orienting experiments on the uptake of uracil-2-^{14}C by the ribonucleic acid of the uracil auxotroph exclude the occurrence of appreciable quantities of 5-fluorocytidylic acid. As shown in Table 2, 5-fluorouracil depresses the incorporation of

Table 1. NUCLEOTIDE COMPOSITION OF RIBONUCLEIC ACID OF *E. coli* STRAINS

No.	Specimen*	Analytical procedure†	Moles per 100 moles nucleotide in ribonucleic acid				
			Adenylic acid	Guanylic acid	Cytidylic acid	Uridylic acid	5-Fluorouridylic acid
1	Strain *B*, normal	I	26·3	29·6	25·1	19·0	—
2	Strain *B*, treated with 5-fluorouracil, 21 hr.	II	23·1	30·4	25·2	11·4	9·8
3	Uracil auxotroph 63–86, normal	III	23·7	32·6	23·0	20·8	—
4	Uracil auxotroph 63–86, treated with 5-fluorouracil, in the presence of uracil, 3 hr.	III	24·6	30·4	24·3	15·9	4·8
5	Uracil auxotroph 63–86, treated with 5-fluorouracil, in the absence of uracil, 16 hr.	II	23·3	32·7	22·5	17·4	4·0

* The figures for No. 1, averages of many determinations, are taken from a previous publication (ref. 8); those for No. 3 are based on several hydrolysis experiments. Specimens No. 2, 4 and 5 were single preparations. Preparations of strain 63–86 treated with 5-fluorouracil, in the presence of uracil, for 1 and 2 hr., gave results very similar to those reported for specimen No. 4.

† The following procedures were employed: (I) chromatography on filter paper in the isobutyrate solvent (ref. 8); (II) separation of nucleotides liberated by sodium hydroxide on 'Dowex-2-formate' columns by gradient elution with formic acid (ref. 3); (III) separation of nucleotides liberated by potassium hydroxide through electrophoresis on filter paper in 0·02 *M* citrate buffer of *p*H 3·5 (ref. 9). In the last-mentioned system uridylic and 5-fluorouridylic acids migrate together. The fluoronucleotide was, therefore, determined separately by electrophoresis in 0·1 *M* borate buffer of *p*H 9·15, in which it migrates as the fastest component (ref. 4), and the estimates of uridylic acid were suitably corrected.

uracil into the uridylic acid, but not its utilization for the cytidylic acid, of the ribonucleic acid of the mutant. It may also be mentioned that we have encountered no indication of the entrance of 5-fluorouracil into the deoxyribonucleic acid of *E. coli*.

Table 2. EFFECT OF 5-FLUOROURACIL ON INCORPORATION OF URACIL-2-^{14}C INTO *E. coli* RIBONUCLEIC ACID

Exponentially growing cells of the *E. coli* mutant 63–86 were washed and starved of uracil (ref. 1). After the addition of uracil-2-^{14}C (20 μgm./ml., specific activity 25,000 c.p.m./μmole) the preparation was divided into two equal parts one of which received 5-fluorouracil (50 μgm./ml.). During the subsequent incubation at 37° C. for 3 hr., equal amounts of ribonucleic acid were formed in both preparations. The radioactivity found in the purine nucleotides and in 5-fluorouridylic acid amounted to less than 5 per cent of that incorporated into the pyrimidine nucleotides. The results are reported per ml. of cell suspension

Specimen	Uracil-2-^{14}C incorporated (μm.moles per ml.)		Incorporation ratio, uridylic acid to cytidylic acid
	Uridylic acid	Cytidylic acid	
Normal	25·1	29·5	0·85
Treated with 5-fluorouracil	14·3	30·0	0·48

We are indebted to Dr. J. A. Aeschlimann, Hoffmann-La Roche, Inc., Nutley, N.J., for specimens of the fluoro compounds used in these studies. The uracil-requiring mutant, originally isolated by Prof. B. D. Davis, of Harvard University, was given us by Dr. E. Borek. We are grateful to Dr. J. J. Saukkonen for helpful discussions and to Mr. R. L. Cooper for technical assistance. The work was supported by grants from the United States Public Health Service and the National Science Foundation.

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MEASUREMENTS OF TEMPORAL ADAPTATION TO SPATIAL DETAIL VISION

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THERE is neurophysiological and psychological evidence[1–3] that temporal as well as spatial adaptation processes are involved in visual perception. From this we argued that after the presentation of a new visual pattern a certain time may be required for a viewer to recognize spatial detail in this new pattern.

A quantitative measure of the perceptual time delay versus the size of detail would be a significant design parameter for a variety of control and communication systems involving the human sense of vision. Tachistoscopic visual recognition tests and reaction time measurements have been reported[4,5], but we think that these have a different significance, because the object, and thus the design, of the experiments are different.

The idea behind our experiments was that, if the viewer requires a definite adaptation time to perceive spatial detail in a newly presented visual scene, it should be possible to measure this effect by presenting the new scene in such a way that the detail size is decreased progressively in time after the instant of presentation. In other words, the new scene is 'blurry' when it is presented to the viewing subject and is made increasingly 'sharper' as function of time. If this temporal increase in objective sharpness occurs faster than the subjective adaptation to the perception of detail, the viewer will not notice the effect. If, however, the temporal adaptation process of the viewing subject proceeds faster than the objective increase in sharpness, it will be noticed by the subject that the scene had been blurred initially. It will be recognized that this approach contains certain elements of transient response testing of electrical systems where the response to a finite transition (in time and amplitude) is given by the

convolution of excitation and transfer function. Hence, for our tests, we argued that the finite temporal increase in sharpness will not be noticed, when the perceptual time response to it is not 'noticeably' slower than what it would have been to a scene which was sharp from the instant of its presentation (response to an ideal stimulus).

Because the 'sharpness' of a picture can be conveniently varied (along the horizontal dimension) by varying the band-width of a television picture signal using a variable low-pass filter, television-type presentation was chosen. This also facilitated rapid change-over from one scene to another by electronic means. The test scenes were stationary (slides) while between presentations of these was displayed an 'interlude' of a normal television programme without sound received from local stations. This, we found, prevented staring at the display area, provided relaxed viewing conditions with no specific bias towards any one fixation point and introduced a close similarity to actual viewing in everyday situations.

The experimental conditions were as follows. Television pictures in accordance with the C.C.I.R. Standard (625/50/25) having a maximum signal band-width of 5 Mc./s. were displayed on a studio monitor of 9 in. × 12 in. picture dimensions. The monitor was surrounded by a flat grey surface, 48 in. × 68 in. Indirect ambient lighting was used in the test area. The brightness of the surround was 0·2 foot-lambert, picture peak white 4 foot-lambert and picture black 0·1 foot-lambert (measured with *SEI*-Photometer). Two different viewing distances were used, one at four times, the other at eight times, picture height. Correspondingly, the maximum picture dimension (width) had a subtended angle of 19° and 9° 30′ for the two distances and minimum detail size for any signal band-width of B Mc./s. is given by $11{\cdot}4/B$ and $5{\cdot}7/B$ minutes of arc respectively.

By means of a voltage-controlled continuously variable low-pass filter[6] the signal band-width was varied from a minimum B_m Mc./s. at the time of scene change-over, $t = 0$, to the system band-width of $B_s = 5$ Mc./s. at $t = T$ in accordance with the following time function:

$$B(t) = B_m \exp\left(\frac{t}{T} \log \frac{B_s}{B_m}\right) \text{ Mc./s., } 0 < t < T$$

Thus the minimum detail size in minutes of arc subtended angle varied for the two viewing distances as:

$$S(t)_4 = \frac{11{\cdot}4}{B(t)} \text{ and } S(t)_8 = \frac{5{\cdot}7}{B(t)}$$

The minimum band-width B_m (degree of blurriness) and the recovery time T as well as the test slide were preset by the experimenter without the subject knowing the conditions. After being prompted by the experimenter the subject pressed a control button by which the interlude scene was replaced by the test scene. The equipment was controlled so that change-over took place during the suppression interval (of approximately 1·5 msec.) between television frames following the pressing of the button. This instant being time $t = 0$ was also the beginning of the recovery of the filter from the preset minimum to full system band-width, which caused the increasing of the objective picture sharpness over the interval $0 < t < T$. Shortly afterwards, the subject announced by 'Yes' or 'No' whether a blurring of the picture was 'seen' or not. The test slide display was then changed back to the interlude by the experimenter before the next experimental condition was established. An average of not more than 150 decisions were made by each subject during a single session in order to prevent tiring. The sequence of the three experimental parameters was selected from a table of random numbers.

In tests of this nature it is desirable to have approximately equal frequencies for 'Yes' and 'No'. We therefore carried out exploratory tests at a viewing distance of four times picture height by which we intended to find values for B_m for which the decisions went from 100 per cent 'No' to 100 per cent 'Yes' when T was varied. For B_m ranging from 0·28 to 4 Mc./s. and T from 20 to 2,600 msec., full transitions from 100 per cent 'No' to 100 per cent 'Yes' could only be obtained for B_m less than 1 Mc./s. At this particular setting the percentage 'No' never fell below 60 per cent and seemed to vary in an unsystematic way for recovery times exceeding 1 sec. We therefore concentrated on the two values of 0·28 and 0·5 Mc./s. for B_m, corresponding to 40·6 and 22·8 minutes of arc for the maximum detail size at four times picture height and 20·3 and 11·4 minutes of arc at eight times picture height viewing distance. The minimum detail size for the two distances and $t > T$ was 2·28 and 1·14 minutes of arc. For each setting of B_m eight different recovery times were used, being $50 < T < 2{,}600$ msec. and $40 < T < 2{,}080$ msec. for $B_m = 0{\cdot}28$ and $B_m = 0{\cdot}5$ Mc./s. respectively.

Three different test slides were displayed. One of these depicted a group of three children, the second an aerial view of a city and the third a stone hut; all three contained fine detail and sharp contours of good contrast but different distribution over the picture area. Since the experiment was not concerned with a semantic recognition, the pictorial content of the slides was of less significance than the availability of detail to which the subject's perception would adapt itself.

Four male subjects belonging to the staff of the Laboratories were tested. They were all familiar with the experiment and the appearance of the reduced detail presentation. Therefore, they may be classed as skilled and critical observers.

The 'percent not seen' ('No') for each experimental condition were computed taking the decisions of all subjects on all test slides together. The resulting

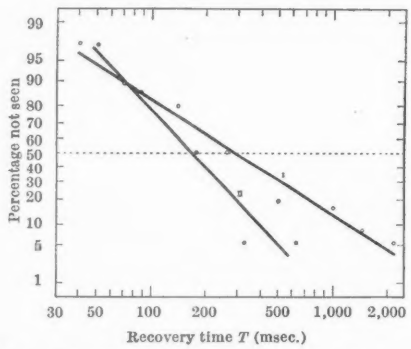

Fig. 1. Percentage not seen versus recovery time. Viewing distance, four times picture height. I, (○ − ○) B_m = 0·5 Mc./s., $S_{max.}$ = 22·8 min. of arc; II, (● − ●), B_m = 0·28 Mc./s., $S_{max.}$ = 40·6 min. of arc

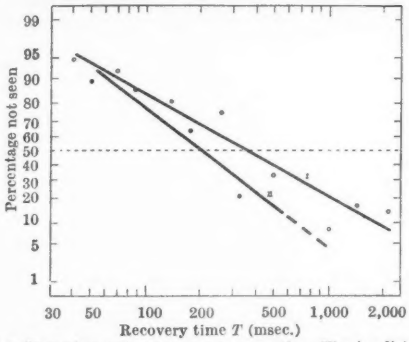

Fig. 2. Percentage not seen versus recovery time. Viewing distance, eight times picture height. I, (○ – ○) B_m = 0·5 Mc./s., $S_{max.}$ = 11·4 min. of arc; II, (● – ●) B_m = 0·28 Mc./s., $S_{max.}$ = 20·3 min. of arc

experimental points were fitted to a normal distribution versus the logarithm of recovery time (T) as shown in Figs. 1 and 2, using the method of least squares. Each point represents 36 decisions. The permissible recovery times for which the initial lack of sharpness in detail was not seen in 50 per cent of the presentations are listed with the respective experimental conditions in Table 1.

Although within the same viewing distance the permissible recovery time increases approximately in the same ratio as the initial detail size decreases, for the doubling of the viewing distance (that is, halving of detail size for the same B_m) the recovery time increases only by a factor 1·25.

From what is known about human sensual phenomena we cannot expect linear behaviour over any extended range of inputs and stimulus conditions. Hence we consider it unjustified to attempt an extrapolation from the restricted data at our disposal at this time. However, the main conclusions which we may draw from the results of the experiment are that a certain time is required after the presentation of a new and remaining visual display before the perception threshold for fine detail is reached, and that due to this effect it is possible to expand the time interval within which detail in changing complex visual displays is offered to the viewer without noticeably interfering with the normal perception process.

Table 1

Viewing distance (picture height)	4 times		8 times	
Minimum detail (min. of arc)	2·28		1·14	
Maximum detail (min. of arc)	40·6	22·8	20·3	11·4
Recovery time (msec.) for 50 per cent 'No'	160	290	200	360

It must be left to further extended experiments to attempt a determination of the functional relationships between the relevant parameters.

We are indebted to the Supervising Engineer, Research, Postmaster-General's Department of Australia, for permission to publish the material contained in this communication.

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EFFECT OF OXYGEN TENSION ON HÆM AND PORPHYRIN BIOSYNTHESIS

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THE adaptive synthesis of cytochromes in aerobic and anaerobic conditions of culture is well known[1]. It has been shown by one of us[2] that as the oxygen tension in the culture medium is increased, the synthesis of cytochrome a_2 by *Aerobacter aerogenes* increases to a maximum, and then decreases when still higher oxygen tensions are applied. The maximum was reached when the oxygen concentration in the medium was of the low order of 0·1 M. It was suggested that a self-regulatory mechanism may operate, the formation of cytochrome a_2 being controlled in accordance with the respiratory requirements imposed by the prevailing oxygen tension.

It occurred to us that prosthetic group synthesis might be affected by the oxygen tension. The prosthetic group of cytochrome a_2 is an iron-chlorin[3], and nothing is yet known about the biosynthesis of this cytochrome or its prosthetic group. It has now been found, however, that the biosynthesis of protoporphyrin and hæm, in chicken erythrocyte preparations *in vitro*, appears to be regulated by oxygen tension.

Whole blood (25 ml.) from normal chickens was shaken with glycine (final concentration 0·056 M) and 1 mgm. each of heparin, penicillin and streptomycin, at 38° C. The gas mixtures all contained 5 per cent carbon dioxide and the relevant concentration of oxygen; nitrogen was used as diluent. The mixtures were made in aspirators, and by displacement with water were bubbled through the incubation mixture at the rate of 2 l./hr. The conical incubation flasks were closed with Bunsen valves, and frothing was controlled by a few drops of octanol. When washed cells were used, 25 ml. of blood was centrifuged and the serum and the 'buffy coat' removed. The erythrocytes were washed three times with isotonic sodium chloride and resuspended in isotonic sodium chloride to a final volume of 25 ml. Substrates and

antibiotics were then added, and incubations carried out, as described for whole blood. Porphyrin and hæm synthesis were determined as described by Drosel and Falk[4].

As shown in Fig. 1, the synthesis of copro- and proto-porphyrins and hæm in this system is regulated by oxygen tension. The synthesis of protoporphyrin and hæm is related linearly to time under these conditions (cf. Fig. 3, and Drosel and Falk[4]).

It is not possible, without further experiments, to say whether or not the optimum oxygen tension for the synthesis of hæm is the same as that for protoporphyrin. The situation is complicated by the effects of oxygen upon the incorporation of iron into protoporphyrin. The tissue-catalysed combination of ferrous iron with protoporphyrin is inhibited by oxygen[6], and the liberation of iron from storage in liver ferritin is promoted by low oxygen tensions[7]. Such factors may contribute to the very great inhibition of hæm synthesis at the highest oxygen tensions (Fig. 1).

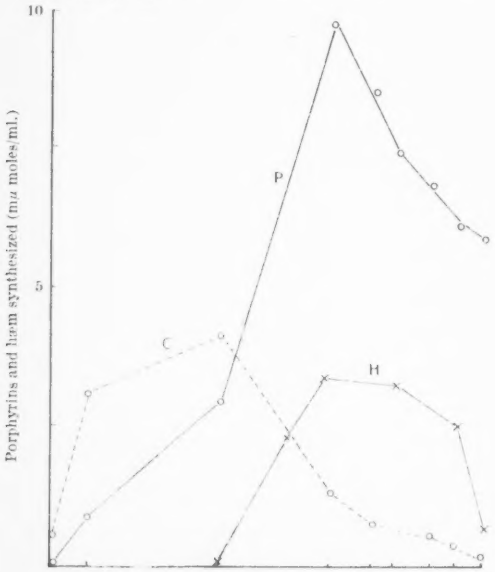

Fig. 1. The control by oxygen of hæm and porphyrin synthesis from glycine in whole chicken blood. Whole blood (25 ml.) was incubated for 8 hr., as described in the text. *P*, protoporphyrin; *C*, coproporphyrin; *H*, hæm

When the erythrocytes were washed three times with isotonic sodium chloride before incubation with glycine, the curve for protoporphyrin formation was found to be similar in form to that shown in Fig. 1, but maximum synthesis was depressed by about 80 per cent (Fig. 2).

That the inhibition of protoporphyrin formation by higher oxygen tensions is reversible is shown in Fig. 3. We have not been able to demonstrate marked degradation of protoporphyrin at 'inhibitory' oxygen tensions.

During the biosynthesis of porphyrins, 'aerobic' reactions are known to be required for the formation of δ-aminolævulic acid, but not again until near the end of the biosynthetic pathway, for the final formation of the vinyl side-chains, and possibly also for the oxidation of a porphyrinogen to the porphyrin level[4,5]. The rising slopes in Fig. 1 reflect these

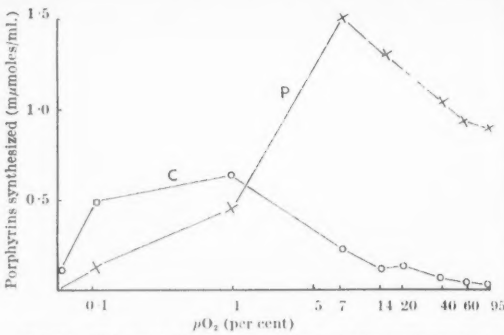

Fig. 2. The control by oxygen of porphyrin synthesis from glycine in washed chicken erythrocytes. The cells from 25 ml. blood were washed and incubated for 10 hr., as described in the text. *C*, coproporphyrin; *P*, protoporphyrin. No protoporphyrin was formed in the complete lack of oxygen

requirements for oxygen; it appears that much lower oxygen tensions suffice for reactions prior to coproporphyrin formation than for the formation of protoporphyrin. If the decreased amounts of protoporphyrin found at higher oxygen tensions are not due to degradation reactions, they would appear to be due to an inhibition by oxygen of some step in the synthetic pathway. As pointed out above, this inhibition is reversible, and it must lie far back in the pathway because coproporphyrin synthesis also is decreased at those high levels of oxygen (Fig. 1) at which protoporphyrin synthesis is decreased.

It appears possible that the control of porphyrin synthesis by oxygen tension may play an important part in cytochrome adaptation in bacteria, though the production of some cytochromes by obligate anaerobes must be considered. There is also evidence[8] that the synthesis by micro-organisms of some colourless enzymes is controlled by oxygen tension.

It is interesting to relate the present findings to animal physiology. The stimulatory effect of anoxia upon erythropoiesis in higher animals[9] and in Crustacea and other invertebrates[10] is well known, and it has also been shown that high tissue-oxygen tensions lead to decreased erythropoiesis[11]. In physiological studies, it has been difficult to separate the effects upon erythropoiesis of oxygen tension from those of endocrinological or other 'plasma'

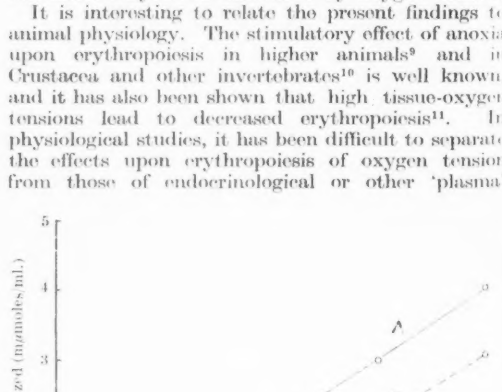

Fig. 3. The reversibility of the inhibition by high oxygen tension of protoporphyrin synthesis. Whole chicken blood was incubated as described in the text. Curve *A*, 5 per cent oxygen; curve *B*, 95 per cent oxygen; curve *C*, 95 per cent oxygen for 4 hr., then 5 per cent oxygen for 4 hr.

factors[9]. The present experiments indicate that there is an oxygen effect exerted directly on a step or steps in the biosynthetic pathway to hæm. In studies *in vitro* of bone marrow from rabbits damaged by X-irradiation, and of normal marrow[12], it has been demonstrated that hæm synthesis precedes that of globin; oxygen tension, by limiting directly the synthesis of hæm, may be expected to limit hæmoglobin synthesis. That the rate of maturation of erythrocyte precursors *in vitro* increases with oxygen tension to a maximum and falls with further increases of oxygen has been demonstrated[13], and it appears possible that maturation of these cells may be linked to hæmoglobin synthesis.

Since the above was written, Lascelles[14] has shown that the formation of bacteriochlorophyll in *Rhodopseudomonas spheroides*, incubated in the dark, reaches a maximum at a pO_2 of about 6 per cent and is depressed at higher oxygen tensions. The formation of δ-aminolævulic acid, and the conversion of this to porphobilinogen, were both found to be sensitive to oxygen tension in *R. spheroides*. We have not investigated the sensitivity of these particular reactions to oxygen in chicken erythrocyte preparations, but we have clear evidence (to be published) that some reaction after the formation of porphobilinogen and prior to the formation of uroporphyrin is inhibited by oxygen tensions higher than about 7 per cent. A preliminary report of this work has been given[15] and it will be described in detail elsewhere. It thus appears that in tetrapyrrole biosynthesis, oxygen may control a number of sensitive steps which could vary both qualitatively and quantitatively from tissue to tissue.

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POSSIBLE ROLE OF HIGHER PROPRIOCEPTIVE CENTRES IN THE PERCEPTION OF VISUAL SPACE AND IN THE CONTROL OF MOTOR BEHAVIOUR

By Dr. B. GUREVITCH

Laboratory of the Physiology of the Visual Analyser, Pavlov Institute of Physiology of the Academy of Sciences of the U.S.S.R., Leningrad

THE variability of oriented motor acts may lead to the assumption that the transformation of proprioceptive impulses into signals for the appropriate regulation of a movement going on greatly depends on temporary changes produced by the conditioning stimulus in the higher centres to which they are flowing. Admitting that orientation in space is mainly based on ontogenetic experience, we are led to suppose that the 'Pavlov feedback'[1]—the temporary connexions of the higher proprioceptive centres—play an important part in the organization of the perception of space.

This view is not generally accepted. Thus, in visual space perception theory, Sherrington's concept[2] identifying the 'muscle sense' of the eyes with the inflow of impulses from the sensory nerve endings in the extrinsic eye muscles is opposed by the generally accepted Helmholtz's theory[3] affirming that we evaluate the direction of our sight on the basis of "the innervation impulses we must apply to transfer the eye in the intended position".

According to a recent suggestion[4], the normal human perception of space "is organized on the conservative principle of a null-hypothesis" and should require no analysis in terms of specific neural events. Such a rather paradoxical view will scarcely satisfy the physiologists interested in the investigation of nervous mechanisms subserving normal life functions.

Now, from Helmholtz's typical 'outflow' theory it would follow that for every eye rotation a corresponding innervation pattern is quantitatively 'predetermined' by the 'sense of innervation'. Hence, as the eyes in their movements (especially horizontal) constitute a system with constant mechanical load, equally directed eye fixation movements of a subject in equal conditions ought to follow the same course.

To study eye rotations under the action of the 'muscle sense', conditioned fixation movements have been systematically investigated in 14 normal subjects. In response to the conditioning sound stimulus (400/sec., 30 db. above audibility threshold, applied during 1½ sec.) the subjects had to turn their eyes in total darkness and to maintain them in the directions in which formerly visible points appeared and were fixed. A total of 14,000 large-scale electrooculographic records were examined, and the results analysed statistically.

Previously, it has been shown[5] that conditioned eye 'muscle sense' has significant directional power, for, in the mean, 40 per cent of all conditioned eye movements were found to be as precisely directed towards the goal as fixation movements to visible objects, and, once attained, a given position of the eyes was further as exactly maintained in complete darkness.

It was no less significantly demonstrated (Fig. 1) that even in a series of successive trials, the saccadic movements of the same subject's conditioned eye fixation, transferring the line of regard into one and the same exact final direction, nevertheless differ essentially one from each other as regards characteristics and composition of the saccade, and namely as

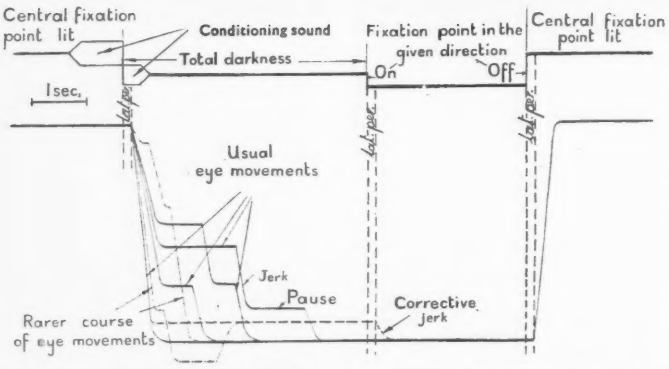

Fig. 1. Representative scheme showing typical variability of equally directed precise (——) or imprecise (– – –) conditioned eye fixation movements in successive trials. Abscissæ: time; ordinates: eye displacement during trials. From more than 2,000 records of conditioned eye movements in the dark of six subjects, in various given directions from 6° 20′ to 35° off the centre in the horizontal plane. Optically elicited and conditioned changes in fixation are preceded by latent periods of about 0·15 sec. lat. per. = latent period

to (*a*) the number of single eye jerks in it (for example, from 1 to 3, and up to 6 jerks in the total saccade), (*b*) the size of these jerks (varying, most generally, from 30 to 100 per cent of the total fixation movement, and in extreme cases from 15 per cent up to 120 per cent of its total size), (*c*) the duration of pauses between successive jerks in the saccades (habitually, from 0·04 to 0·7 sec., and up to 1·92 sec.), (*d*) the angular speed of the eye movement in jerks of equal size (from 60 to 140 per cent, and not infrequently up to 200 per cent of its mean value). In some cases reversals have been observed in the usual succession of diminishing jerks, and in some other cases excessive jerks have been recorded, followed by corrective jerks in the opposite direction.

Such a fundamental variability of the composition of equally directed saccadic fixation movements characterizes conditioned eye rotations of every size in the horizontal plane as well as in vertical and in diagonal planes. We may conclude that conditioned eye movements of equal total size and direction, just like motor acts of the organism as a whole, are generally produced by essentially variable sequences of innervation impulses, which is inconsistent with the innervation theory. On the other hand, the now well-known proprioceptors[6] of the extrinsic eye muscles are the only sensory organs firing during eye rotations in total darkness, and this proprioceptive feedback is used to bring the position of the eyes into accordance with the temporary central changes brought about by the conditioning stimulus. Thus the observations strongly support the hypothesis that the 'muscle sense' of the eyes is mainly controlled by temporary changes in the higher proprioceptive centres, and it must be noted that such a mechanism would form a physiological basis for visual illusions arising in cases of dissociation between the actual eye position and the evaluation of the line of regard, which in fact depends on conditioned fixation reflex training.

Because of the restriction of nerve connexions engaged and the constancy of mechanical load during movement, the conditioned eye fixation reflex may be considered as a simple physiological model of more complicated motor behaviour acts.

It appears that to remove apparent contradictions between 'peripheral' theories of behaviour control (like Sherrington's) and 'central' theories (like Helmholtz's), Pavlov's theory must be applied to sensory arcs of the conditioned reflex. Fig. 2 represents a hypothetical scheme based on Pavlov's concept of temporary cerebral kinæsthetic feedback. The external stimuli, having formerly coincided with reactions fulfilled and re-inforced, on eliciting a reaction by the way of its effectory centre, simultaneously produce in the higher proprioceptive centres changes just like those which, beforehand, became reinforced at the fulfilment of the reactions. During the course of the reaction, the impulses ascending to the higher proprioceptive centres from the sensitive endings in the effectory organs are constantly re-codified according to the functional state of these centres and, being thus transformed into signals of correction, take part in the regulation of the reaction until its fulfilment and repeated reinforcement. Thus, it appears that behaviour acts remain on the whole adequately directed, because of the regulation during their course of the efferent nerve supply by the higher proprioceptive centres, according to changes brought to these centres by conditioning stimuli.

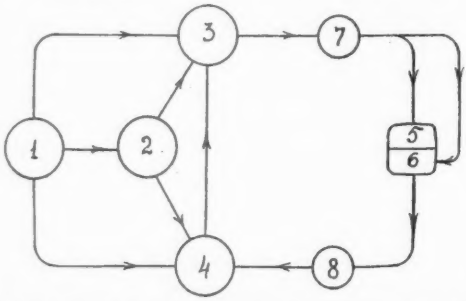

Fig. 2. Hypothetical scheme of the central 'sensory' control of behaviour; 1, cortical representation of the conditioning stimulus; 2, cortical centre of the unconditioned reaction; 3, cortical effectory and 4, cortical sensory centre of the conditioned reaction; 5, effectory organ and 6, sensory endings in it; 7, 8, subcortical centres. Arrows show the supposed directions of the main innervation flows engaged

It is only in the frame of the conditioned changes in the higher sensory centres that the afferent impulses from the effectory organs get their full meaning for the organism. 'Muscle sense' arises from co-operation and mutual control, as behaviour acts are going on, of certain sensory events of external and internal origin, this co-operation and mutual control being rendered possible by the conditioned reflex mechanisms. Thus, the mechanisms of elaboration of temporary changes in the higher proprioceptive centres may be regarded as probably the more general physiological basis of psychic space perception phenomena.

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 30

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 2.30 p.m.—Anniversary Meeting.

INSTITUTE OF METAL FINISHING (in the Canterbury Room of the Charing Cross Hotel, London, W.C.2), at 2.45 p.m.—Mr. A. A. B. Harvey: "The Role of the Scientific Society" (Presidential Address).

UNIVERSITY COLLEGE (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Prof. E. P. Kennedy (University of Chicago): "The Biosynthesis of Complex Lipids".* (First of two lectures in Biochemistry. Further lecture on December 7.)

ROYAL INSTITUTION, LIBRARY CIRCLE (at 21 Albemarle Street, London, W.1), at 5.30 p.m.—Dr. L. Pearce Williams: "Faraday Through His Manuscripts".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 8.30 p.m.—Prof. C. von Fürer-Haimendorf: "Sherpas of Eastern Nepal".

Tuesday, December 1

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Prof. R. E. D. Bishop: "Vibration Problems in Engineering".*

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND ELECTRONICS SECTIONS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Dr. L. Essen, Mr. J. V. L. Parry and Mr. J. McA. Steele: "Frequency Variations of Quartz Oscillators and the Earth's Rotation in Terms of the N.P.L. Cæsium Standard".

UNIVERSITY OF LONDON (at Imperial College of Science and Technology, London, S.W.7), at 5.30 p.m.—Prof. H. K. Porter: "Physiology has No Frontiers" (Inaugural Lecture).

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. J. L. Gowans: "The Lymphocyte".* (Twelfth of fifteen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation. Further lectures on December 3, 8, 10.)

PLASTICS INSTITUTE (at the Wellcome Building, 183–193 Euston Road, London, N.W.1), at 6.30 p.m.—Mr. M. R. E. Ashenden: "Plastics and the Law".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Dr. I. M. Hall: "Transonic Flow Over Swept Wings".

Wednesday, December 2

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. J. Marechal and Mr. P. de Radzitsky: "Potentialities of Urea in Dewaxing Middle and Heavy Distillates".

INSTITUTE OF INFORMATION SCIENTISTS (at the Berners Hotel, 10 Berners Street, London, W.1), at 6 p.m.—Discussion on "Languages in Information Work—To What Extent is Competence in a Foreign Language an Essential Qualification for an Information Scientist?".

Wednesday, December 2—Thursday, December 3

IRON AND STEEL INSTITUTE (in the Great Hall, Caxton Hall, Caxton Street, London, S.W.1, and the Hoare Memorial Hall, Church House, Great Smith Street, London, S.W.1), at 9.30 a.m. daily—Autumn General Meeting.

Thursday, December 3

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Mr. P. R. Bell: "The Origin of Indian Corn".*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Mr. F. H. C. Edgecombe and Prof. R. G. W. Norrish, F.R.S.: "A Study of the Mechanism of Photochemical Electron Transfer Processes in Solution"; Mr. I. M. Dawson and Mr. E. A. C. Follett: "An Electron Microscope Study of Synthetic Graphite".

INSTITUTE OF MARINE ENGINEERS (joint meeting with the INSTITUTE OF NAVAL ARCHITECTS, in the Weir Hall, 10 Upper Belgrave Street, London, S.W.1) at 4.45 p.m.—Prof. G. Aertssen: "New Sea Trials on the Sandblasted *Lubumbashi*".

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, London, W.C.2), at 5 p.m.—Dr. E. R. Leach: "Rethinking Anthropology"* (Malinowski Memorial Lecture).

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Mrs. Mildred Valley Thornton: "Indians of British Columbia".

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. C. B. B. Wood and Mr. I. J. Shelley: "The Transmission of News Film over the Trans-Atlantic Cable".

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (joint meeting with the SOCIETY FOR APPLIED BACTERIOLOGY, at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 6.15 p.m.—Dr. F. Brown: "Infective Ribonucleic Acid from the Virus of Foot-and-Mouth Disease".

Friday, December 4

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Nuclear Magnetic Resonance", opened by Dr. N. Sheppard and Dr. R. E. Richards.

SOCIETY OF DYERS AND COLOURISTS (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 6 p.m.—Mr. R. C. Oakley: "Dyeing of Ribbons"; Mr. R. Woods: "Dyeing of Carpet Yarns".

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Dr. H. A. Thomas: "Electronic 'Brains'".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

LECTURERS OR ASSISTANT LECTURERS (suitably qualified graduates, with some experience in any of the branches of electrical engineering) in the Department of Electrical Engineering—The Registrar, The University, Manchester 13 (November 21).

LECTURER IN TELECOMMUNICATION, to undertake undergraduate and postgraduate teaching and to supervise research involving information analysis and experimental work, including the human element—Head of the Electrical Engineering Department, Imperial College of Science and Technology, Exhibition Road, London, S.W.7 (November 24).

LECTURER (with a degree in psychology or equivalent and experience of teaching and clinical work) IN EDUCATIONAL PSYCHOLOGY—The Registrar, University College, Swansea (November 28).

SENIOR LECTURER AND A LECTURER (preferably with a major interest in applied thermodynamics or applied mechanics, especially the theory of machines) in Engineering (Mechanical)—The Registrar, The University, Manchester 13 (November 28).

SENIOR LECTURER, LECTURER, and an ASSISTANT LECTURER IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Registrar, Royal Technical College, Salford, Lancs (November 28).

ASSISTANT LECTURER (DEMONSTRATOR) IN ANATOMY, to undertake teaching and research; and a RESEARCH ASSISTANT (with an honours degree in zoology or medical sciences, and preferably a knowledge of German) IN THE DEPARTMENT OF ANATOMY, for work mainly on the central nervous system—The Secretary, University College London, Gower Street, London, W.C.1 (November 30).

LECTURER IN APPLIED MATHEMATICS at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, November 30).

RESEARCH OFFICER (with veterinary qualifications), for studies on virus of foot-and-mouth disease in farm animals—The Secretary, Research Institute (Animal Virus Diseases), Pirbright, Surrey (November 30).

SENIOR LECTURER IN CHEMISTRY at Victoria University of Wellington, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, November 30).

RESEARCH ASSISTANT (with a first- or second-class honours degree in chemistry, or alternatively, graduate member of the Royal Institute of Chemistry) IN CHEMISTRY—The Principal, Coventry Technical College, Butts, Coventry (December 1).

LECTURER (with experience in either heavy electrical machinery or of power transmission and distribution) IN ELECTRICAL ENGINEERING—The Registrar, University College of South Wales and Monmouthshire, Cathays Park, Cardiff (December 4).

ASSISTANT OR ASSOCIATE PROFESSOR OF APPLIED MATHEMATICS—The Chairman, Department of Mathematics, McMaster University, Hamilton, Canada (December 10).

SENIOR LECTURER, or LECTURER (Grade I) IN BOTANY at University College, Ibadan, Nigeria—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 12).

SENIOR LECTURER or LECTURER IN EXPERIMENTAL PHARMACOLOGY—The Secretary, The University, Aberdeen (December 14).

CHAIR OF PHYSICS, CHAIR OF GEOLOGY, CHAIR OF ZOOLOGY, and CHAIR OF BACTERIOLOGY in the University of Khartoum—The Registrar, University of Khartoum, c/o Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 15).

EMPIRE RHEUMATISM COUNCIL FELLOW (with medical or scientific qualifications), to prosecute research in the field of rheumatism—The General Secretary, Empire Rheumatism Council, Faraday House, 8–10 Charing Cross Road, London, W.C.2 (December 15).

ASSISTANT LECTURER or LECTURER IN THE DEPARTMENT OF ZOOLOGY—The Registrar, The University, Liverpool (December 19).

LECTURER or ASSISTANT LECTURER IN PHARMACEUTICS at the University of Malaya (Singapore Division)—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (January 15).

IMPERIAL CHEMICAL INDUSTRIES RESEARCH FELLOWS IN CHEMISTRY, PHYSICS, METALLURGY, ENGINEERING, GEOLOGY, AGRICULTURAL SCIENCE and related subjects—The Registrar of the University of Wales, University Registry, Cathays Park, Cardiff (March 7).

ASSISTANT BIOCHEMIST (Basic grade) (with an appropriate science degree, or Associate or Graduate Member of the Royal Institute of Chemistry) IN THE DEPARTMENT OF PATHOLOGY—The Superintendent, Northampton General Hospital, Northampton.

ASSISTANT, Grade B, IN PHYSICS—The Clerk to the Governors, Woolwich Polytechnic, London, S.E.18.

HEAD OF THE DEPARTMENT OF AERONAUTICAL AND MECHANICAL DESIGN—The Registrar, Hatfield Technical College, Hatfield, Herts.

MASTER TO TEACH MATHEMATICS, with a good share in sixth form work—The Headmaster, Bedford School, Bedford.

MASTER TO TEACH SCIENCE AND MATHEMATICS up to Middle School level—The Headmaster, King's School, Rochester, Kent.

RESEARCH ASSISTANT TO WORK IN THE PHARMACOLOGY DEPARTMENT on inflammation and anti-inflammatory substances—The Dean, Guy's Hospital Medical School, London Bridge, London, S.E.1.

SENIOR ASSISTANT PHYSICIST (with some experience in medical physics), to act as deputy to the Principal Physicist and to take responsibility in radiation physics and isotope work—The House Governor, King's College Hospital, Denmark Hill, London, S.E.5.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Council for the Preservation of Rural England. Thirty-third Annual Report, 1958–1959. Pp. 76+4 plates. (London: Council for the Preservation of Rural England, 1959.) [810

Tobacco Manufacturers' Standing Committee. Research Papers. No. 4: Cigarette Smoke Condensate—Preparation and Routine Laboratory Estimation. By H. R. Bentley and J. G. Burgan. Pp. i+9. (London: Tobacco Manufacturers' Standing Committee, 1959.) [810

E.M.I. News, Vol. 1 No. 1 (October 1959.) (The Newspaper of the E.M.I. Group of Companies.) Pp. 12. (Hayes, Middx.: Electric and Musical Industries, Ltd., 1959.) [810

British Society for the Promotion of Vegetable Research. Ninth Annual Report, 1958 (October 1957–September 1958.) Pp. viii+55. (Wellesbourne, Warwick: British Society for the Promotion of Vegetable Research, 1959.) [810

British Museum (Natural History). The Neolithic Revolution. By Sonia Cole. Pp. vi+60+18 plates. (London: British Museum (Natural History) 1959.) 5*s*. [810

Colonial Office. The Colonial Territories, 1958–1959. Pp. xxv+199. (Cmnd. 780.) (London: H.M. Stationery Office, 1959.) 10*s*. 6*d*. net [810

Post Office. Report of the Advisory Committee on the Inland Telegraph Service, 1958. Pp. iii+11. (London: H.M. Stationery Office, 1959.) 1*s*. net. [810

Report of the Committee on the Rating of Charities and Kindred Bodies. Pp. iv+86. (Cmnd. 831). (London: H.M. Stationery Office, 1959.) 5*s*. net. [810

Air Ministry: Meteorological Office. Tables of Temperature, Relative Humidity and Precipitation for the World. Part 3: Europe and the Atlantic Ocean North of 35° N. Pp. x+159. (London: H.M. Stationery Office, 1959.) 14*s*. net. [810

British Scientific Instrument Research Association. Research Report M.39: Adhesives Guide. By Mrs. Joyce Hurd. Pp. viii+138. (Chislehurst: British Scientific Instrument Research Association, 1959.) 20*s*. [810

National Central Library. 43rd Annual Report of the Executive Committee for the year ending 28 February 1959. Pp. 20. (London: National Central Library, 1959.) [810

Scientific Council for Africa South of the Sahara. Publication No. 28: Radio Isotopes (Pretoria, 1957). Pp. 230. Publication No. 34: C.S.A. Specialists' Meeting on Road Research, Lourenco Marques, 1959. Pp. 48. (London: Scientific Council for Africa South of the Sahara, 1959.) [810

Fifth Report from the Select Committee on Estimates, Session 1958–59: United Kingdom Atomic Energy Authority (Production Group and Development and Engineering Group.) Pp. lxxxv. (London: H.M. Stationery Office, 1959.) 5*s*. net. [1410

University of London. University College Calendar, 1959–60. Pp. lxvi+502. (London: University College, 1959.) [1410

Effects of Printing Types and Formats on the Comprehension of Scientific Journals. By E. C. Poulton. Pp. ii+22. (Cambridge: At the University Press, 1959.) [1410

Department of Scientific and Industrial Research: Building Research Station. National Building Studies—Special Report No. 29: Organization of Building Sites. By R. C. Sansom. (European Productivity Agency Project No. 302/1). Pp. x+186+20 plates. (London: H.M. Stationery Office, 1959.) 21*s*. net. [1410

Department of Scientific and Industrial Research. Problems of Progress in Industry—4: What They Read and Why—The Use of Technical Literature in the Electrical and Electronics Industries. By Nigel Calder. Pp. 24. (London: H.M. Stationery Office, 1959.) 2*s*. [1410

General Register Office. The Registrar General's Quarterly Return for England and Wales—Births, Deaths and Marriages; Infectious Diseases; Weather; Population Estimates, quarter ended 30th June 1959. (No. 442, 2nd quarter, 1959.) Pp. 27. (London: H.M. Stationery Office, 1959.) 2*s*. net. [1410

Other Countries

Fisheries Research Board of Canada. Bulletin No. 120: Redfish Distribution in the North Atlantic. By Wilfred Templeman. Pp. viii+173. (Ottawa: Queen's Printer, 1959.) 1.75 dollars. [810

Epilepsia, Vol. 1, No. 1 (March 1959). Fourth Series. (Journal of the International League Against Epilepsy.) Pp. iv+116. 4 issues to the volume, approx. 480 pages per volume. Subscription price: 57*s*.; 8 dollars, or 30 D.fl. per volume (post free). (Amsterdam: Elsevier Publishing Company, 1959.) [810

Chemotherapy: a Symposium held at the Central Drug Research Institute, Lucknow, November 2–4, 1958. Pp. xi+176. (New Delhi: Council of Scientific and Industrial Research, 1959.) [810

Uganda Protectorate. Annual Report of the Forest Department for the year ended 31st December, 1958. Pp. viii+80+4 plates. (Entebbe: Government Printer, 1959.) Shs. 6. [810

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LETTERS TO THE EDITORS

CHEMISTRY

Photolysis of Thionine In Rigid Medium—Measurement by Spectrofluorimetry

THE quantum efficiency of photo-decomposition in rigid solvents at liquid nitrogen temperature is for many substances quite low, and it is customary to expose the photolyte directly to the concentrated beam from a mercury lamp to produce sufficient photo-product for the measurement of its absorption spectrum. When measuring the quantum efficiency, it is difficult to produce a sufficiently high intensity of monochromatic light at a series of wave-lengths and covering the whole area of the reaction cell. A further difficulty is the absorption of the irradiating light by the products of the reaction. This can produce a large inner filter error if comparatively high concentrations are photolysed to a significant degree. With thionine in E.P.A. glass the quantum efficiency is less than 0·005 and to avoid these difficulties the application of spectrofluorimetry was investigated. As an analytical technique, spectrofluorimetry is much more sensitive than absorption spectroscopy[1] and allows the use of dilute solutions, so that a comparatively small proportion of the photolysing light is absorbed. A further advantage is that light from a monochromator can be focused on a small area of the reaction cell so that much higher intensities can be obtained than if the whole cell face were illuminated. After irradiation, comparison of the intensity of the red thionine fluorescence from the irradiated area with that from unirradiated areas on either side gives a direct measure of the proportion of dyestuff consumed. The spectrofluorimeter can also be used to observe the fluorescence emission and excitation spectra of fluorescent photo-products. At the concentrations used ($< 10^{-5}$ *M*), complications due to energy transfer processes are negligible.

The spectrofluorimeter previously described[1] was used both for photolysis with monochromatic light and for fluorescence measurements. The cuvette was replaced by a quartz Dewar vessel having two windows in line for the passage of the irradiating beam and for absorption measurements and one window in a position at right angles to the irradiating beam for the observation of the fluorescence. The reaction cell had a square face, 2·9 cm. × 2·9 cm. and an optical depth of 0·5 cm., the fluorescence being observed through the flat edge of the cell. The exciting light was chopped at 800 c./s. and the fluorescence was detected by means of an 800 c./s. tuned amplifier so that phosphorescence of duration greater than a few msec. was rejected.

The quantum efficiencies for the photodecomposition of thionine in ether–ethanol–*iso*pentane glass (2 : 2 : 1) were found to be as in Table 1.

The quantum efficiency is low at all wave-lengths observed. It is highest at 248 mμ, on the high-frequency side of the second absorption band of thionine. At 578 mμ, in the visible absorption band, there was no detectable decomposition, the quantum yield being less than one thousandth of that at 248 mμ. This wave-length effect thus runs parallel to that observed in the flash photolysis of dilute solutions of thionine at room temperature[2] where a long-lived species, tentatively identified as the semi-thionine free radical, was observed when ultra-violet light was used.

Table 1. QUANTUM EFFICIENCY FOR THE PHOTOLYSIS OF THIONINE. (Ether–ethanol–*iso*pentane glass at 77° K.)

Wave-length (mμ)	Dose-rate incident on reaction cell (micro-einstein per min.)	Time of irradiation (min.)	Quantum efficiency × 10^3
578	0·33	120	< 0·002
578	0·34	240	< 0·001
302	0·032	270	0·04
302	0·11	285	0·04
283	0·009	240	0·67
283	0·028	120	0·58
248	0·012	270	2·0
248	0·027	100	3·0
248	0·053	40	2·9

The fluorescence emission spectra of the photolysed glasses showed a band with maximum at 510 mμ. The corresponding excitation spectra showed three well-defined maxima at 260, 320 and 455 mμ. The absorption spectra of the photo-products, obtained by photolysing larger concentrations of thionine (10^{-4}–10^{-5} *M*) showed well-defined peaks at 410, 428 and 635 mμ, none of which corresponded to the excitation maximum, and it thus appeared that more than one product of photolysis could be obtained. Measurements of the thionine recovered after melting the irradiated glass, and also after aeration of the resulting solution, suggested that about 50 per cent of the photolysed dyestuff was converted into the semiquinone free radical.

A detailed account of these experiments will be published elsewhere.

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BIOCHEMISTRY

Monamycin: a New Antibiotic

IN 1944 Meredith reported[1] the results of an extensive screening programme designed to select soil micro-organisms with antagonism towards *Fusarium oxysporum cubense*, the causative agent of the Panama disease of the banana plant. An examination of a selection of Meredith's cultures has led to the separation, from a mixed culture, of a new species which we have named *Streptomyces jamaicensis*. The species grows readily on a medium containing neopeptone and glucose both in stationary and in aerated, submerged cultures to produce an antibiotic which is distributed in the culture medium and the mycelium.

This antibiotic has been isolated by a procedure which includes the following essential stages. The crude product is extracted from the culture fluid and the mycelium with ether or butanol. The extract, after removal of solvent, is concentrated with respect to antibiotic by two countercurrent distributions using, in turn, the systems ethyl acetate, cyclohexane,

methanol, water (12 : 10 : 10 : 7) and light petroleum (boiling point 60–80° C.), methanol, water (10 : 10 : 1) followed by chromatography using the ion exchange resin 'Amberlite' *C.G.*45. The product is crystallized from light petroleum as needles, melting point 126° C. This compound to which we have assigned the name monamycin, has properties which distinguish it from known antibiotics.

Monamycin is a base which gives a crystalline monohydrochloride [melting point 187° C., $[\alpha]_D^{18} - 62 \pm 5°$ (*c.* 0·9 in ethanol)]. Analysis results are in good agreement with those required by the molecular formula $C_{22}H_{36-38}N_4O_5$ with one N-methyl and three C-methyl groups. The ultra-violet spectra of the base and its salts exhibit only end absorption. The infra-red spectrum of monamycin shows no evidence of aromatic character but suggests the presence of an amide linkage. It does not react with sodium metaperiodate or with hydrogen in the presence of platinum catalyst.

Monamycin is active at high dilution against a variety of Gram-positive organisms, including strains of *Staphylococcus aureus* which are resistant to penicillin, aureomycin, chloramphenicol and sulphamethazine. There is no significant activity against any of the Gram-negative organisms which have been examined. Table 1 shows the activity towards typical organisms

Table 1

Organism	Highest effective dilution (gm. ml.)
Staphylococcus aureus (A.T.C.C. 9144)	1/20,000,000
Bacillus subtilis (A.T.C.C. 6633)	1/15,000,000
Streptococcus pyogenes (N.C.T.C. 8198)	1/10,000,000
Streptomyces lavendulae	1/8,000,000
Escherichia coli	<1/100,000
Pseudomonas fluorescens	<1/100,000

in terms of the highest effective dilution as measured by the agar-streak method[2].

This antibiotic is a relatively stable compound. There is no loss of activity after autoclaving in aqueous solution at *p*H 9 for 10 min. at 114° C., but losses occur at *p*H values lower than 7. It is not inactivated by human serum. Acute toxicity studies involving injection into mice by the subcutaneous route showed no unfavourable reactions at a dosage of 850 mgm./kgm. This was the highest dose tested.

We are grateful to Miss L. Wong and Mrs. S. Smith for technical assistance, to the Tropical Products Institute for financial support and to both the National Research Development Corporation and the Microbiological Research Establishment (Ministry of Supply), Porton, for facilitating and undertaking the larger-scale production of monamycin.

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Transplantation Immunity: Separation of Antigenic Components from Isolated Nuclei

Extraction of transplantation antigens, introduced and substantially improved by Billingham, Brent and Medawar[1,2] has always been performed by exposing the cells to ultrasonic oscillations in distilled water or solutions of low ionic strength. We here report results obtained with less drastic techniques more usual in biochemistry. The test for antigenicity is based on the ability of active components to provoke a 'second set' reaction in a skin homograft of the donor strain[1].

All extraction procedures are conducted in the cold. Thymus and spleen nuclei isolated according to Billingham *et al.*[1], represent a suitable and constant basic material. They are extracted four times with 0·14 *M* sodium chloride, 0·01 *M* sodium citrate, *p*H 7, in a Waring blendor, for 75 sec. each time. The 4 supernatants, after centrifugation for 10 min. at 15,000 *g*, are collected for subsequent manipulation. The residue, mostly deoxyribonucleoprotein, is still slightly active after four such treatments, but has no detectable activity after six extractions. If allowed to stand for some time, the collected supernatants show a faint opalescence which may be cleared by centrifuging at 15,000 *g* for 30 min. The sediment, which seems to contain some kind of deoxyribonucleic acid-protein complex, is antigenically active, but the greater part of the antigenic activity remains in solution.

Various purification procedures may then be applied. Antigen may be precipitated by lowering the *p*H to 5. Fractional precipitation shows the best yields to occur between *p*H 7 and 6 and between *p*H 6 and 5·5. The precipitate, most of it consisting of ribonucleoprotein also contains hæmoglobin which is a regular contaminant of our nuclei suspensions. Though it dissolves most of the ribonucleoprotein, citrated saline does not bring back the antigen into solution. This insoluble fraction is highly antigenic and represents a useful preparation for many purposes.

Alternatively, antigen may be precipitated from the initial supernatants by ammonium sulphate up to 50 per cent saturation. The sediment is completely soluble in citrated saline. This procedure eliminates most of the hæmoglobin which precipitates at 60–80 per cent saturation. This method has the advantage of securing soluble antigenic matter which may be easily handled for analysis, particularly by chromatography and electrophoresis. Selective chromatography on calcium phosphate has shown the identity of the antigen with one of the first peaks. This probably represents a very high degree of purification.

Enzymic assays have been performed, very often with the insoluble fraction after precipitation at *p*H 5. Deoxyribonuclease, ribonuclease, trypsin, actinomycetin F_1.*B.* and lyzozyme were tested. Only the last enzyme significantly reduced the antigenic activity. However, this is probably due to a non-specific complexing effect, for it was not accompanied by a corresponding increase in reducing sugars. In other experiments enzymic assays were combined with separation procedures. Although they permitted a higher degree of purification, they failed to solve the fundamental problems of the chemical nature of the antigen, which, according to the latest hypothesis of Billingham, Brent and Medawar[2], seems to be a complex polysaccharide of comparatively low molecular weight. This and other problems are discussed in detail elsewhere.

It is of interest to note that a toxic component may be extracted from the nuclei. Constantly recovered from the same fractions, it seems to be a glycoprotein.

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Electrophoretic Heterogeneity of Trypsin

IN a previous communication[1] it was reported that crystalline trypsin, when submitted to paper electrophoresis at *p*H 2·6, shows the presence of three different and proteolitically active fractions, even in the absence of calcium ions.

The present work was undertaken to investigate further the electrophoretic behaviour of crystalline trypsin and of the trypsin fractions separated by paper electrophoresis.

Our results seem to indicate that the behaviour of crystalline trypsin in free boundary electrophoresis at *p*H 2·5 is fundamentally similar to that obtained with paper electrophoresis. They show also that the fractions separated by paper electrophoresis at *p*H 2·6 bear no relation to those separated by free electrophoresis at *p*H 5 in the presence of calcium ions. These facts are interpreted as resulting from the presence in crystalline trypsin of at least four electrophoretic components.

The crystalline trypsin and the technique used for paper electrophoresis were the same as described previously[1]. For preparative purposes, however, sheets of Whatmann No. 3 paper measuring 32 cm. × 54 cm. were used. The localization of the trypsin fractions was obtained by dyeing three guiding strips, one cut from the middle and one from each side of the sheet. The part of the paper containing the main trypsin fraction was cut and extracted with 3 ml. of 0·001 *M* hydrochloric acid. This extract was dialysed for 48 hr. against two changes of the proper buffer solution and then submitted to free electrophoresis in a Perkin Elmer Model 38 instrument.

Two buffers were used in these experiments: a glycine (0·05 *M*), hydrochloric acid (0·025 *M*) and sodium chloride (0·05 *M*) buffer of *p*H 2·5 and a sodium acetate (0·04 *M*) and acetic acid (0·04 *M*) buffer of *p*H 5; to these solutions calcium chloride up to a concentration of 0·04 *M* was added when necessary.

The results of the analyses, by free electrophoresis, of the main component of crystalline trypsin separated by paper electrophoresis at *p*H 2·6 are given in Table 1. They show that this component is homogeneous when analysed by free electrophoresis at *p*H 5, at *p*H 2·6 in the presence of calcium ions and also by paper electrophoresis at *p*H 2·6. However, by free electrophoresis at *p*H 5 in the presence of calcium ions two fractions were obtained. These results indicate that trypsin contains at least four distinct electrophoretic fractions, namely, F_1 and F_2 migrating together as the main component during paper electrophoresis at *p*H 2·6, and separated only when in the presence of calcium at *p*H 5 by free electrophoresis; and fractions F_3 and F_4 which are resolved directly by paper electrophoresis at *p*H 2·6. Another point that emerges from these experiments is that the fractions obtained by paper electrophoresis at *p*H 2·6 seems to bear no relation to those shown by free electrophoresis at *p*H 5 in the presence of calcium.

The experiments of fractionation of crystalline trypsin by free electrophoresis are shown in Table 2. Fraction E_4 is present in crystalline trypsin and amounts to about 3 per cent of the total proteins; this component, however, is precipitated almost entirely during dialysis against *p*H 5 buffer, and is therefore absent from the electrophoretic diagrams obtained at *p*H 5. Adding the precipitate F_4 to crystalline trypsin resulted in an increase in the amount of the slower fractions which is separated by free electrophoresis at *p*H 2·5. Since F_1 and F_2 are not separated at this *p*H either by paper electrophoresis or by free electrophoresis (Table 1), we may conclude that F_4 at this *p*H, migrates together with F_3 during free electrophoresis. The composition of the fractions in this case, namely, free electrophoresis at *p*H 2·5, are considered to be $(F_1 + E_2)$ for the faster, and $(F_3 + F_4)$ for the slower one, this result being independent of the presence of calcium ions.

At *p*H 5, F_4 is absent, and in the presence of calcium ions, F_1 is separated from F_2. The ratio between the percentages of F_1 and F_2—found after fractionation at this *p*H—of the main paper electrophoretic component, indicates that F_3 migrates together with F_1 and not with F_2. Accordingly, the components of the fractions observed at *p*H 5 in the presence of calcium ions should be $(F_1 + F_3)$ for the faster, and F_2 for the slower fraction.

It is necessary, however, to point out that no conclusive evidence exists for identifying F_2, obtained by direct electrophoresis of crystalline trypsin, with F_2 resulting from the fractionation of the main component separated by paper electrophoresis, inasmuch as their mobilities are different. We have not found satisfactory explanation for this result beside admitting further heterogeneity of these three fractions.

Another point of interest is the pronounced effect of calcium ions on the mobility of both trypsin fractions during electrophoresis at *p*H 2·5, indicating that at

Table 1. ELECTROPHORETIC ANALYSIS OF THE MAIN COMPONENT $(F_1 + F_2)$* OF CRYSTALLINE TRYPSIN

Fractions obtained by paper electrophoresis of crystalline trypsin $(F_1 + F_2 + F_3 + F_4)$, at *p*H 2·6			
	(F_3) (14·7 per cent)		
	$(F_1 + F_2)$ (82·7 per cent)	Paper electrophoresis at *p*H 2·6 .. .. one fraction	$(F_1 + F_2)$
		Free electrophoresis at *p*H 5 + Ca^{++} .. two fractions	(F_1) (68 per cent, $\mu = 4{\cdot}36$) (F_2) (32 per cent, $\mu = 3{\cdot}95$)
		Free electrophoresis at *p*H 2·5 + Ca^{++} .. one fraction	$(F_1 + F_2)$ ($\mu = 5{\cdot}93$)
		Free electrophoresis at *p*H 5 .. .. one fraction	$(F_1 + F_2)$ $\mu = 5{\cdot}1$)
	(F_4) (2·6 per cent)		

* The probable composition of each electrophoretic fraction is given inside brackets.

Table 2. Free Boundary Electrophoresis of Crystalline Trypsin

Buffer*	*p*H 5	*p*H 5 + Ca^{++}		*p*H 2·5		*p*H 2·5 + Ca^{++}	
Probable composition of electrophoretic fractions	$(F_1 + F_2 + F_3)$§	$(F_1 + F_3)$	(F_2)	$(F_1 + F_2)$	$(F_3 + F_4)$	$(F_1 + F_2)$	$(F_3 + F_4)$
Mobility†	5·08	4·97	4·56	7·66	5·81	6·09	4·37
Percentage Composition‡	100	77	23	77·5	22·5	78	22

* See text for the composition of the buffers.
† Mobility $\times 10^{-5}$ of the ascending boundary.
‡ Based on the areas of the ascending boundary.
§ See text for explanation.

this *p*H, as should be expected, both fractions are reacting with calcium ions.

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Inhibition of Reduced Diphosphopyridine Nucleotide Oxidation by Ammonia

The toxic responses of plants to ammonia supplied as anhydrous or aqueous ammonia or ammonium salts are well documented[1-3]; but relatively little is known regarding the means by which toxicity to either plant or animal cells is brought about[4]. In the course of an investigation of this problem it was found that ammonia strongly inhibits the exogenous utilization of glucose, pyruvate, citrate, α-ketoglutarate, succinate and malate by a number of intact tissues and particulate preparations from plant sources. Of the Krebs' cycle substrates, succinate was inhibited less by ammonia than the others used. This partial resistance of succinate oxidation to ammonia inhibition pointed toward a possible effect on diphosphopyridine nucleotide since this does not serve as a co-factor for succinate oxidation. To investigate this question further, studies were made of the effect of ammonia on the reduction and oxidation of diphosphopyridine nucleotide in homogenates of red beet-root, using the 340 mμ absorption peak of the reduced diphosphopyridine nucleotide. Homogenates of the fresh root of red beet (*Beta vulgaris*) were prepared in the cold by hand grinding and centrifugation. The suspending medium was 0·2 *M tris* (hydroxymethyl) aminomethane buffer at *p*H 8·5 containing 1·0 *M* sucrose and 5×10^{-3} *M* ethylenediamine tetra acetic acid.

The reduction of diphosphopyridine nucleotide was carried out in a preparation containing 0·2 ml. of the homogenate; diphosphopyridine nucleotide, 3 μ*M*, malate, 3 μ*M*; and 0·5 *M tris* buffer to make 3 ml. The oxidation of reduced diphosphopyridine nucleotide was measured in a preparation containing a similar amount of homogenate; adenosine diphosphate, 3 μ*M*; potassium ascorbate, 15 μ*M*; and reduced diphosphopyridine nucleotide, 0·6 μ*M* in a total volume of 3 ml. The substrate and co-factors were prepared fresh daily from commercial materials. A limited amount of reduced diphosphopyridine nucleotide oxidation was demonstrated by the homogenate without the addition of either adenosine diphosphate or ascorbate; but the presence of these materials, particularly ascorbate[5], greatly enhanced the rate obtained.

Changes in the absorbance of this preparation at 340 mμ were followed by means of a Beckman model *DU* spectrophotometer fitted with a recording attachment. Maximum rates of reaction and the most reproducible results were obtained when the homogenates were supplied with either nitrogen or oxygen during the measurements. For this purpose a capillary tube drawn to an extremely fine orifice was inserted into the silica cell. For studies of diphosphopyridine nucleotide reduction, nitrogen gas was passed through this tube and for oxidation of reduced diphophosphopyridine nucleotide, oxygen was used. Ammonia gas obtained from an atmosphere in equilibrium with 4 *M* ammonium was supplied through another tube, with either oxygen or nitrogen used as a carrier for the ammonia.

The inhibitory effect of ammonia on reduced diphosphopyridine nucleotide oxidation is shown in Fig. 1. The reversible nature of this inhibition is demonstrated by the resumption of oxidation upon removal of the ammonia supply. This recovery of activity also indicates that a form of ammonia in close equilibrium with ammonia is the effective agent of inhibition, since when the supply of ammonia is stopped, oxidation is quickly resumed as the ammonia is washed out of solution by the aerating oxygen supply. The results in Fig. 1 also show that *p*H in this range has relatively little effect on the rate of reduced oxidation diphosphopyridine nucleotide. Measurements made with a similar preparation under the same conditions showed that from an initial *p*H of 8·4, during the first minute of treatment with ammonia, the *p*H rose to 8·6, which was maintained until the second 2-min. application which resulted in a rise to the final *p*H of 9·1.

The increase in the rate of oxidation of reduced diphosphopyridine nucleotide shown by the preparation treated with ammonia after removal of the first application of ammonia has been consistently found in a number of experiments. It is possible that this might be due to an effect of the altered *p*H of the medium on the reaction, but when the *p*H is changed over the same range by additions of potassium hydrox-

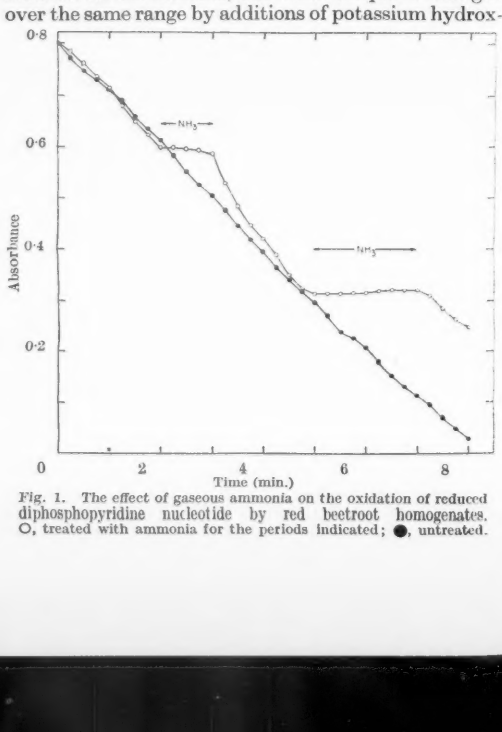

Fig. 1. The effect of gaseous ammonia on the oxidation of reduced diphosphopyridine nucleotide by red beetroot homogenates. ○, treated with ammonia for the periods indicated; ●, untreated.

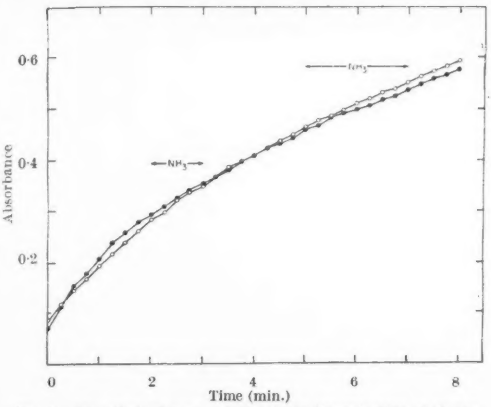

Fig. 2. The effect of gaseous ammonia on the reduction of diphosphopyridine nucleotide by red beetroot homogenates. ○, treated with ammonia for the periods indicated; ●, untreated.

ide, no such stimulation is found. Another possibility is that some intermediate accumulates during the ammonia inhibition period and upon removal of the inhibitor this intermediate is converted to diphosphopyridine nucleotide at a rate faster than the overall reduced diphosphopyridine nucleotide > diphosphopyridine nucleotide reaction.

The specificity of ammonia inhibition for oxidation of reduced diphosphopyridine nucleotide is illustrated by Fig. 2, which shows the reduction of this substance by two preparations of beetroot, one of which was treated with ammonia at the times indicated. It may be seen that exposure to ammonia has little effect on the rate of the reaction in this experiment.

The means by which ammonia interferes with electron transport in the diphphosphopyridine nucleotide system of beetroot homogenates is not clear. Ammonia may be serving as an uncoupling agent for oxidative phosphorylation; but the present evidence does not clearly show this to be the case. The rapid equilibrium which can exist between ammonia and ammonium suggests that the forms may in some way serve as a preferential hydrogen donor and thus be 'sparing' reduced diphosphopyridine nucleotide. It is also possible that ammonia is competing with the hydrogen ion during oxidation of reduced diphosphopyridine nucleotide, or with this substance itself for a site on the oxidative enzyme.

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Binding of the Sulphydryl Group in D-Amino-Acid Oxidase Apo-Protein with Flavin Adenine Dinucleotide

The importance for the enzymatic activity, of the sulphydryl group in D-amino-acid oxidase apoprotein has been reported by several authors[1-4], but their conclusions concerning the binding site of the sulphydryl group differed. Singer and Barron[1] and Singer[2] suggested that the protein sulphydryl group might be involved directly in the binding of the substrate. Frisell and Hellerman[3] concluded, however, that the enzyme sulphydryl group is not needed for the direct binding of the substrate, a D-amino-acid. Kubo *et al.*[4] considered that the protein sulphydryl group binds with the imino NH(3) group of flavin adenine dinucleotide.

In our laboratory, the binding between D-amino-acid oxidase apo-protein and the coenzyme flavin adenine dinucleotide has been studied in detail[5,6], but a decision as to the exact binding of the protein sulphydryl group is still needed. For this purpose, a detailed kinetic analysis of the mechanism of inhibition by *p*-chloromercuribenzoate was carried out.

The enzyme protein and flavin adenine dinucleotide were purified by the methods of Negelein and Brömel[7] and that of Yagi *et al.*[8], respectively. The oxidase activity was measured with a conventional Warburg manometer.

It was confirmed[3,4] that *p*-chloromercuribenzoate inhibits the oxidase interfering with both the substrate and flavin adenine dinucleotide. Since our recent results[6] demonstrated that the phenyl carboxyl group is essential for the competition of benzene derivatives with the substrate and that it does not affect the binding of flavin adenine dinucleotide with the oxidase protein, it may be concluded that the inhibitory action of *p*-chloromercuribenzoate connected with the substrate is due to its phenyl carboxyl group, as Frisell and Hellerman supposed[3], and that the inhibitory action of *p*-chloromercuribenzoate connected with flavin adenine dinucleotide is due to its property of reacting with the sulphydryl group.

The dissociation constant of flavin adenine dinucleotide with the protein (K_f) was calculated to be $1{\cdot}1\times10^{-7}$ M using the Michaelis-Menten equation. The reaction velocity of the oxidase (v) in the presence of a concentration (i) of inhibitor, which competes with flavin adenine dinucleotide for the protein, can be shown to be:

$$v=\frac{Vf}{K_f(1+i/K)+f} \qquad (1)$$

where f is the rate-limiting concentration of flavin adenine dinucleotide, V is the maximum velocity obtained in the presence of excess flavin adenine dinucleotide, and K is the dissociation constant of the inhibitor combining with the protein in competition with flavin adenine dinucleotide.

Lineweaver-Burk plots in the presence of $2{\cdot}8\times10^{-7}M$ *p*-chloromercuribenzoate, the rate-limiting concentration of flavin adenine dinucleotide, and excess DL-alanine ($0{\cdot}15$ M) are on a straight line with the intercept $1/V$. From the slope of this line, K was calculated to be $1{\cdot}6\times10^{-7}$ M.

From equation (1) and the Michaelis-Menten equation, the following equation can be derived:

$$\frac{v_0}{v}=1+\left\{1-\frac{v_0}{V}\right\}\frac{i}{K} \qquad (2)$$

where v_0 and v are the reaction velocities in the absence and presence of the inhibitor. Measured values of v_0/v plotted against the concentrations of *p*-chloromercuribenzoate were on a straight line with intercept I, as shown in Fig. 1, curve III. The value of K obtained from the slope of this line agreed with that found above.

These results show that *p*-chloromercuribenzoate combines with the sulphydryl group of the oxidase protein in competition with flavin adenine dinucleotide. The question then arises of whether

p-chloromercuribenzoate competes with the riboflavin monophosphate part or the adenylic acid part of flavin adenine dinucleotide, in other words: Which part of flavin adenine dinucleotide actually combines with the protein sulphydryl group? To solve this problem, we devised a kinetic method using riboflavin-5′-monosulphate and adenosine-5′-monosulphate which, respectively[8], compete specifically with the riboflavin monophosphate part and the adenylic acid part of flavin adenine dinucleotide. The specific inhibitors were synthesized by the methods of Takahashi, Yagi and Egami[9] and of Egami and Takahashi[10], respectively.

Assuming that the reaction mixture contains two inhibitors, one of which competes with the riboflavin monophosphate part of flavin adenine dinucleotide, and the other with the adenylic acid part (case I), the reaction velocity can be shown to be:

$$v = \frac{Vf}{Kf(1+i_1/K_1+i_2/K_2+i_1i_2/K_1K_3)+f} \text{ or}$$

$$v = \frac{Vf}{Kf(1+i_1/K_1+i_2/K_2+i_1i_2/K_2K_4)+f} \quad (3)$$

where i_1, i_2 are the concentrations of two inhibitors, and K_1, K_2 are the dissociation constants of the complexes (i_1-enzyme protein) and (i_2-enzyme protein), respectively. K_3 is the dissociation constant for (i_1, i_2-enzyme protein) $\rightleftharpoons i_2 +$ (i_1-enzyme protein), and K_4 is that for (i_1, i_2-enzyme protein) $\rightleftharpoons i_1 +$ (i_2-enzyme protein).

On the other hand, if the reaction mixture contains two inhibitors both of which compete with the same part of flavin adenine dinucleotide, (case II), the reaction velocity can be shown to be:

$$v = \frac{Vf}{Kf(1+i_1/K_1+i_2/K_2)+f} \quad (4)$$

From the Michaelis-Menten equations and (3) or (4), equations (5) and (6) can be derived.

These formulæ show that the plots v_0/v against the concentrations of inhibitors give a second-order curve in case I and a straight line in case II.

$$\frac{v_0}{v} = 1 + \left\{1 - \frac{v_0}{V}\right\}\left\{\frac{i_1}{K_1}+\frac{i_2}{K_2}+\frac{i_1i_2}{K_1K_3}\right\} \text{ or}$$

$$\frac{v_0}{v} = 1 + \left\{1 - \frac{v_0}{V}\right\}\left\{\frac{i_1}{K_1}+\frac{i_2}{K_2}+\frac{i_1i_2}{K_2K_4}\right\} \quad (5)$$

$$\frac{v_0}{v} = 1 + \left\{1 - \frac{v_0}{V}\right\}\left\{\frac{i_1}{K_1}+\frac{i_2}{K_2}\right\} \quad (6)$$

In the actual measurements of the inhibition by *p*-chloromercuribenzoate and riboflavin-5′-monosulphate, v_0/v plotted against the concentrations of both inhibitors gave a second-order curve as shown in Fig. 1, curve V, whereas *p*-chloromercuribenzoate and adenosine-5′-monosulphate gave a straight line (Fig. 1, curve IV).

From these results, it may be concluded that *p*-chloromercuribenzoate combines with the protein in competition with the adenylic acid part of flavin adenine dinucleotide. Thus, it may be supposed that the protein sulphydryl group combines with the adenylic acid part of flavin adenine dinucleotide, most probably with the amino group of its adenine nucleus.

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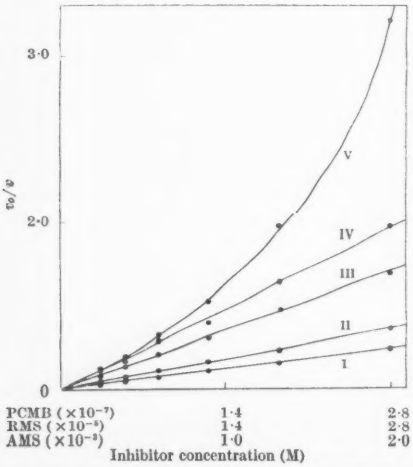

Fig. 1. The inhibitory action of *p*-chloromercuribenzoate on D-amino-acid oxidase. The reaction mixtures contained 15 μgm. of the oxidase protein; excess DL-alanine (0·15 moles); flavin adenine dinucleotide ($1·7\times10^{-7}$ moles); and graded concentrations of inhibitors. I, Adenosine-5′-monosulphate (AMS); II, Riboflavin-5′-monosulphate (RMS); III, *p*-Chloromercuribenzoate (PCMB); IV, *p*-Chloromercuribenzoate and adenosine-5′-monosulphate; V, *p*-Chloromercuribenzoate and riboflavin-5′-monosulphate. v was 132 μl. oxygen uptake/30 min. and v_0 was 80 μl. oxygen uptake/30 min.

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Lack of Direct Effect of Erythropoietin on Human Erythroid Cells *in vitro*

The existence in the plasma and urine of anæmic patients and animals, of a substance which is capable of increasing the rate of production of red cells in normal recipients is now an undisputed fact. However, the mechanism by which this substance exerts its stimulatory effect is still obscure. Two mechanisms for its action can be postulated. A direct action of the hormone on erythroid cells to stimulate rate of cell division as well as rate of differentiation, that is to say, hæmoglobinization, would increase the rate of turnover of marrow. Alternatively the hormone could accelerate the rate of differentiation of stem cells into the erythron to expand the volume of active marrow without affecting the rate of turnover.

By application of the techniques of auto-radiography to bone marrow cells *in vitro*, the first hypothesis was investigated. The rate of synthesis of deoxyribonucleic acid and the length of the cell cycle were measured with ^{3}H-thymidine or ^{14}C-formate, the rate of hæmoglobin synthesis with iron-59. The methods and their application have been described previously[1].

Urinary erythropoietin from the urine of a patient with aplastic anæmia was used. The residue from

processing a quantity of normal urine was used as an inactive control substance. The marrow samples which were used were obtained from patients in the course of normal diagnostic investigations.

A number of treatment schedules were tried. The pre-treatment with erythropoietin ranged from 0 to 23 hr. before addition of the label, but in no case were significant differences found between cultures treated with active and inactive urinary preparations. Table 1 summarizes the results and shows that neither the grain count per cell, an index of the rate of synthesis of dioxyribonucleic acid, nor per cent labelled cells, a measure of the ratio of the length of the period of synthesis of deoxyribonucleic acid to the length of the generation time, is affected. In order to give an impression as to the character of the distribution of the grain counts, the mean, the median, and the maximum or highest observed grain count are given.

Table 1. Effects of Human Urinary Erythropoietin on DNA Synthesis and Cell Cycle in Human Bone Marrow Cultures
Auto-radiographic estimation of radio-activity in basophilic normoblasts after incubation with either active or inactive urinary erythropoietin. ^{3}H-thymidine (0·25 μc./ml. medium) or ^{14}C-formate (1·25 μc./ml. medium), as deoxyribonucleic acid label, added at times shown.

Incubation time	Grain counts/Labelled cell Mean	Median	Maximum	Percentage of cells labelled
6 hr.		Active		
(30 μgm./ml.)*	16	8	90	78
^{3}H-thymidine		Inactive		
added at 0 hr.	14	8	64	74
5 hr.		Active		
(30 μgm./ml.)	7	4	37	68
^{3}H-thymidine		Inactive		
added at 4 hr.	10	8	49	66
8 hr.		Active		
(15 μgm./ml.)	10	8	80	69
^{3}H-thymidine		Inactive		
added at 2 hr.	12	7	80	67
24 hr.		Active		
(60 μgm./ml.)	36	24	120	90
^{3}H-thymidine		Inactive		
added at 23 hr.	36	20	90	89
24 hr.		Active		
(60 μgm./ml.)	68	62	164	69
^{14}C-formate		Inactive		
added at 22 hr.	68	59	145	70

* Concentration of active or inactive urinary preparation added at zero time

Table 2. Effect of Human Urinary Erythropoietin on the Incorporation of Iron-59 in Human Bone Marrow Culture
Bone marrow cultures were treated for the times shown with active or inactive urinary erythropoietin preparations and with 0·25 μc./ml. of iron-59. Cultures were run in triplicate. Separation, washing, plating and counting techniques are described elsewhere[1]

Incubation	Iron-59 added at time (hr.)	Counts/min./ml. of final washed cell suspension Erythropoietin	Inactive
4 hr. with 30 μgm./ml.*	0 hr.	2147	2280
12 hr. with 60 μgm./ml.	6 hr.	1451	1219
18 hr. with 125 μgm./ml.	5 hr.	4230	3360

* Concentration of active or inactive urinary preparation added at zero time.

In Table 2 are shown the radio-activity determinations on washed cells after exposure to iron-59. In this case also there is no significant difference between the incorporation by the control or by cultures treated with erythropoietin. In one experiment in which iron-59 was added at 6 hr. to a 24-hr. culture, auto-radiographic analysis of the iron incorporation into basophilic normoblasts was undertaken. The mean grain count for erythropoietin treated cells was 185, control cells had a mean grain count of 192. Again no direct action could be demonstrated.

It must be concluded from these observations that erythropoietin has no observable direct actions on the two processes measured, namely, relationships of deoxyribonucleic acid synthesis and cell cycle, and hæmoglobin synthesis. An aliquot of the preparation used in bone marrow culture was assayed by the measurement of iron incorporation in starved rats according to the technique of Hodgson[2], and the urinary preparation from the aplastic anæmic patient was found to be highly active. The injection into 200-gm. rats of 2·5 mgm. twice daily for two days produced a three-fold increase in the incorporation of a tracer dose of iron-59. The normal urine preparation was inactive. As the dose used *in vivo* was 50 μgm./gm. of body-weight, the dose used in culture for approximately 5×10^6 cells was of equivalent size or higher.

Recently Schroeder, Gurney and Wackman[3] reported that 'anæmic plasma' increased radio iron incorporation as much as four-fold in bone marrow suspensions. Our observations do not substantiate their conclusions that erythropoietin stimulates hæmoglobin synthesis *in vitro*. It is felt, however, that their failure to control the final specific activity of the isotope in the culture medium provides the explanation for their results.

We cannot exclude the possibility that the urinary erythropoietin is not identical with plasmaerythropoietin, and that hydrolysis of a conjugated product is necessary for activity of the urinary product. Gordon[4] reported, however, that the urinary erythropoietin is active in his isolated hind-limb preparation. Unless tissue esterases which would be capable of liberating an active product are present in the hind limb, a conjugated form would not be active.

As a consequence, we feel that stimulation of red cell production with erythropoietin is effected not through direct action on nucleated red cells, but by some other mechanism. Evidence for a mechanism involving the increased rate of differentiation of primitive stem cells into the early erythroid population of cells has been described elsewhere[5].

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A Cardiolipin-like Compound in Rat Liver Mitochondria

Marinetti *et al.*[1] found that rat liver mitochondria readily incorporate radioactive orthophosphate into a phosphatidic acid-like polymer. They have further suggested[2] that this compound may be important in oxidative phosphorylation.

In the course of a study of the lipids of rat liver cell organelles, we have found in the lipids of rat liver mitochondria (extracted with chloroform methanol 2:1 (v/v)), a fraction which is eluted off a silicic acid chromatography column[3] with chloroform-methanol 7:1 (v/v) and which resembles in many respects the cardiolipin first isolated from ox heart by Pangborn[4]

and more recently studied by Gray and MacFarlane[5]. This fraction constituted 7 per cent of the mitochondrial lipid carboxylic acid esters and 10 per cent of the lipid phosphorus recovered from the silicic acid column. Amino nitrogen was virtually absent from his fraction, the amino nitrogen phosphate molar ratio being less than 0·01. The glycerol/phosphate/carboxylic ester molar ratio was 1·4:1:1·5 or 1·4:1:2·1 when the ester estimation was performed on the methyl esters of the fatty acids prepared by interesterification of the intact lipid[6]. This latter ratio suggests a compound containing 4 glycerols, 3 phosphates and 4–6 fatty acids. Gray and MacFarlane found values of 3:2:4; Taylor and McKibbin[7] obtained values of 3:2:3 for a similar lipid isolated by them from dog liver phospholipids and Pangborn[8] reported a 4:3 glycerol/phosphate ratio.

The methyl esters of the fatty acids of this fraction prepared as indicated above, were analyzed by gas liquid chromatography using both 'Apiezon *L*' and an adipate ester of polyethylene glycol as stationary phases. The results (together with those of Gray and MacFarlane for comparison), are presented in Table 1 using the fatty acid notation suggested by Ahrens *et al.*[9], as percentages of the total fatty acid methyl esters.

Table 1

	Present work (rat liver mitochondria)		Gray and MacFarlane (whole ox heart)
C12—15	0·66		0
C16— 0 (palmitic)	1·99		0·49
C16— 1 (palmitoleic)	1·93		5·23
C17— ?	0·20		1·22
C18— 0 (stearic)	0·43		0·79
C18— 1 (oleic)	11·93		11·0
C18— 2 (linoleic)	79·5		80·0
C20— 4 (arachidonic)	0·71		0·74
C20— 3	1·22	2·77	
C20— 2	0·762		0·74
C22— 6	0·79		
Total saturated	3·3		2·5
Total unsaturated	96·84		97·7

It is remarkable that compounds of such similar composition, even with respect to the major fatty acids, should have been found in two such different sources. The rat brain contains hardly any of this compound, at least with this fatty acid composition (L. A. Biran, unpublished from this laboratory). While the relation of this fraction to the phosphatidic acid-like polymer of Marinetti *et al.* (they reported an ester/phosphate molar ratio of 6·04) is not clear, it would be of considerable interest to determine the turnover of both the phosphate and fatty acid moieties of this lipid.

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A Reduced Triphosphopyridine Nucleotide-linked Cystine Reductase in the Clothes Moth, *Tineola bisselliella* (Humm)

In the course of studies on the digestion of wool by insects we have examined the clothes moth for the presence of reductases of disulphide bonds. Enzyme preparations were made by cold homogenization of whole larvæ of the clothes moth followed by centrifugation at 30,000 *g* for 30 min. and dialysis of the supernatant for 20 hr. against cold 0·05 *M tris* (hydroxymethyl) aminomethane-hydrochloric acid buffer at *p*H 7·3. Cystine reductase activity was demonstrated by measuring the decrease in absorption at 340 mμ of reduced triphosphopyridine nucleotide (California Foundation) using anærobic cuvettes in a Beckman *DU* spectrophotometer. Also the production of SH-groups was measured by a modified Grunert and Phillips colorimetric nitroprusside method[1], and confirmed by titration with phenyl mercuric nitrate.

The decrease in absorption at 340 mμ due to oxidation of reduced triphosphopyridine nucleotide in the presence of cystine is rapid compared with the control without cystine (Fig. 1). A slight decrease in the control even under the anærobic conditions used may be explained by the presence of endogenous substrates in the insect extract which are not removed during the preparation. The production of SH-groups by the enzyme in the absence of cystine is negligible, however the addition of cystine and di- or tri-phosphopyridine nucleotide causes a slight increase in SH-groups. Some activation is caused by reduced di- but considerably more by reduced triphosphopyridine nucleotide. High reductase activity follows the addition of a substrate for a triphosphopyridine nucleotide-linked dehydrogenase (glu-

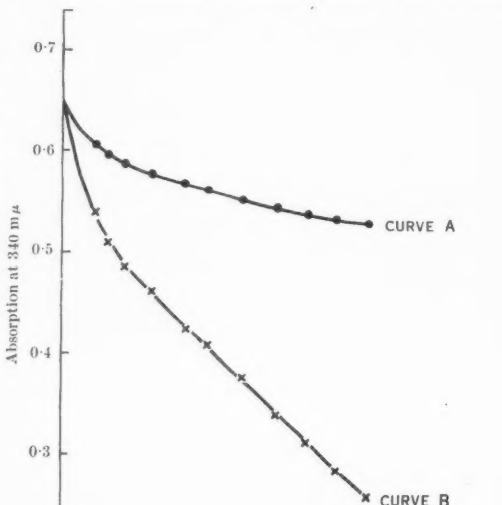

Fig. 1. Effect of 1-cystine on oxidation of reduced triphosphopyridine nucleotide by dialysed *Tineola* extract. Reaction mixture for curves *A* and *B* contained 1 ml. extract, 270 μmoles *tris* (hydroxymethyl)—aminomethane (*p*H 7·3 with hydrochloric acid), 0·34 μmoles reduced triphosphopyridine nucleotide in a final volume of 3·2 ml. The mixture for curve *B* contained, in addition, 2·1 μmoles 1-cystine. The reaction was carried out in anærobic Beckman cuvettes.

Table 1. CYSTINE REDUCTASE IN DIALYSED *Tineola* ENZYME PREPARATION

Reaction mixture	Cystine reductase activity (μ moles SH-groups produced)
Enzyme alone	0·06
" +cystine	0·36
" " +DPN	0·48
" " +TPN	0·48
" " +DPNH	0·76
" " +TPNH	1·64
" " +G-6-P	2·76
" " +G-6-P+TPN	4·10

Reaction mixtures: 1 ml. enzyme, 125 μmoles tris (hydroxymethyl)—aminomethane (*p*H 7·3 with hydrochloric acid), diluted to final volume 2·5 ml. and containing the following where indicated: 4·2 μmoles l-cystine, 3·2 μmoles glucose-6-phosphate (G-6-P), 0·07 μmoles triphosphopyridine nucleotide, 0·07 μmoles diphosphopyridine nucleotide, 1 μmole reduced triphosphopyridine nucleotide, 1 μmole reduced diphosphopyridine nucleotide. Incubated for 1 hr. at 25°C. under anærobic conditions in Thunberg tubes. SH-group estimations by the colorimetric nitroprusside method.

cose 6-phosphate) which, in the presence of added triphosphopyridine nucleotide yields the highest activity observed (Table 1). Since addition of glucose-6-phosphate alone activates the cystine reductase it appears that triphosphopyridine nucleotide is not completely removed by the dialysis under the conditions employed. Enzymic reduction of triphosphopyridine nucleotide by glucose-6-phosphate, *iso*citrate and malate, and of diphosphopyridine nucleotide by malate has been demonstrated spectrophotometrically in these *Tineola* preparations. These dehydrogenase activities were retained on storage but the cystine reductase activity was lost under the same conditions.

Other disulphide bond reductases are also present in the insect preparation, for example, cystine reduced diphosphopyridine nucleotide (Table 1), glutathione reduced triphosphopyridine nucleotide and glutathione reduced diphosphopyridine nucleotide reductase but these are all of relatively low activity. Glutathione tri-[2] and di-phosphopyridine nucleotide[3] reductase activities have been described in plants and the reduced triphosphopyridine nucleotide-linked enzyme in animal tissues[4]. The reduced triphosphopyridine nucleotide-linked cystine reductase, which has not previously been described, may be compared with similar enzymes from other sources which are reduced diphosphopyridine nucleotide specific[5,6]. It is not possible from the present work to say whether the activity with reduced tri- and reduced di-phosphopyridine nucleotide is due to different enzymes or to the same enzyme having different specificity for the pyridine nucleotide coenzymes.

It is thought that these enzymes, particularly the cystine-reduced triphosphopyridine nucleotide reductase, are involved in the process of digestion of wool by clothes moths and other insects. Cystine is an important component of wool and it is known that wool which has a proportion of its disulphide bonds reduced becomes more easily digestible[7].

Full details of this work will appear elsewhere.

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Production of Ethylene by Mitochondria from Tomatoes

EVOLUTION of ethylene by ripening fruit and acceleration of ripening by application of the gas to green fruit has been of interest for many years. However, little more than the gross aspects of these phenomena has been known until recently, when Burg and Thimann[1] used gas chromatography to study the evolution of ethylene by apple-tissue slices. In our laboratories we have been able to observe the production of ethylene by a mitochondrial fraction from tomatoes.

All steps in the preparation of the mitochondria were conducted at 0–1°C. Since the *p*H of whole ground tomatoes is about 4, rapid neutralization is necessary during preparation of the homogenate. This was best accomplished by grinding tomatoes with an equal weight of phosphate buffer (0·5 *M* potassium dihydrogen phosphate, 0·25 *M* sucrose, adjusted to *p*H 8·1 with sodium hydroxide) at low speed in a Waring blendor. The homogenate was filtered through cheesecloth and the filtrate centrifuged at 4,000 *g* for 7 minutes to remove cell fragments. The supernatant was then centrifuged at 35,000 *g* for 10 minutes. (A force of 15,700 *g* was sufficient to separate the mitochondria, but 35,000 *g*, which separated no additional particles, packed the mitochondria into easily handled pellets.) These were suspended in 0·5 *M* sucrose-0·01 *M* phosphate buffer of *p*H 7 by means of a Servall 'Omnimixer', and recentrifuged at 35,000 *g* for 10 min. The washed mitochondria were then suspended in buffer-substrate mixture (0·5 *M* sucrose, 0·125 *M* potassium dihydrogen phosphate, 10^{-3} *M* magnesium sulphate, 10^{-3} *M* manganese sulphate, $1{\cdot}98\times10^{-3}$ *M* adenosine triphosphate, 0·25 *M* malic acid, $3{\cdot}3\times10^{-4}$ *M* diphosphopyridine nucleotide, *p*H 7·0).

This mitochondrial suspension was used for determination of the rate of production of ethylene by a method described previously[2], the only difference being that, in order to prevent contamination by micro-organisms, the air stream entering the respiration chamber was passed through columns of glycerol on glass wool and cotton, and the respiration chamber and stopper were sterilized prior to use. (All buffer solutions used in the preparation of the mitochondrial fraction were also sterile, except that adenosine triphosphate and diphosphopyridine nucleotide were added after sterilization.) No evidence of growth of micro-organisms was obtained when nutrient agar and Pratt's medium[3] were inoculated with the mitochondria-substrate mixture.

The ethylene-producing system appeared to be relatively stable, for after 26 hr. storage of the mitochondria-substrate mixture at 0–1°C. it produced ethylene at about one-half the original rate.

Typical results are presented in Table 1. Comparisons are made with ethylene production by whole fruit of the same variety, determined previously[4].

No ethylene was detectable from mitochondria from green tomatoes or those in the early stages of ripening. Maximum production of this gas occurred

Table 1. PRODUCTION OF ETHYLENE BY *V*-121 TOMATOES (WHOLE FRUIT AND MITOCHONDRIAL FRACTIONS) AT VARIOUS STAGES OF MATURITY

Stage of ripeness	Ethylene (μl./kgm./4 hr.) Whole fruit	Mitochondria
Mature green	2·5	None detected
Medium turning	10	4
Advanced turning	12	11
Firm ripe	6	8

with mitochondria from tomatoes in the advanced turning stage (one-half to three-quarters of the surface red). Production of ethylene by mitochondria appears to follow the pattern obtained with the whole fruit, where evolution of ethylene reaches a peak at the 'advanced turning' stage and then decreases to low amounts as the fruit reaches full ripeness.

I acknowledge the technical assistance of T. A. Tribe.

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Duration of Protective Action of Interferon Against Infection with West Nile Virus

Inactivated influenza virus has been shown to inhibit the growth of western equine encephalitis virus in eggs[1] and in the mouse brain[2] and it seemed likely, therefore, that interferon, which appears to mediate viral interference[3] should also inhibit the growth of viruses of this group. We have found that interferon strongly inhibits the growth of West Nile virus in cultures of chick fibroblasts, and our colleague Dr. J. S. Porterfield has shown[4] that it prevents plaque formation by a number of 'arbor' viruses, including West Nile and yellow fever viruses. In this paper we describe experiments carried out with West Nile virus, on the duration of the protective action of interferon.

Cell suspensions were prepared from 10-day old chick embryos by a slight modification of the trypsinization technique described by Dulbecco[5]. Test tubes were planted with 5×10^6 cells in 1 ml. of Gey's buffered salt solution plus 0·5 per cent lactalbumin hydrolysate, and kept stationary at 37°C. Six tubes were used for each experimental group. After 24 hr. each tube received an addition of 0·5 ml. of a dilution of West Nile virus (Egypt 101 strain) containing $3·2\times10^5$ plaque-forming units, from a capillary stock of mouse-brain virus kept at −78°C. The medium was changed daily or every second day and each fluid was titrated individually for viral hæmagglutinin by diluting it serially in borate buffer at *p*H 9·0, and adding an equal volume of a 0·25 per cent suspension of goose erythrocytes in phosphate buffer to give a final *p*H of 6·6. At the same time, cultures were examined by low-power microscopy and the degree of cell degeneration noted. In cultures of this kind large yields of viral hæmagglutinin were produced within the first 3 or 4 days' incubation (Fig. 1, curve *A*) when the cells degenerated completely.

Similar cultures were then set up except that interferon was incorporated in the initial medium. The interferon was prepared by incubating irradiated influenza virus with chick chorio-allantoic membranes and the amount used was sufficient to reduce the yield of the *PR*8 strain of influenza virus to less than 6 per cent of the controls in the assay described by Lindenmann, Burke and Isaacs[6]. The medium was changed daily or every second day but no further interferon was added. In one experiment where the medium was changed daily the cells remained healthy in appearance and the medium showed a daily fall in *p*H, for 11 days, when cell degeneration commenced. Throughout this period no viral hæmagglutinin was produced. (Controls showed that interferon did not inhibit viral hæmagglutination.) In other experiments where the medium was changed either daily or every second day only 2 tubes out of 78 examined showed the presence of hæmagglutinin on the seventh day; the others showed no hæmagglutinin when examined repeatedly over the period of 3–11 days after the initiation of viral infection. It seems therefore that when the cells are suspended in a simple maintenance medium a single dose of interferon given before the start of infection protects them from West Nile virus infection for almost the whole of their life-time.

When similar experiments were carried out with medium enriched by the addition of 5 per cent calf serum and 1·5 per cent chick embryo extract, viral hæmagglutinin production and interference similar to that described above were noted during the first 2 or 3 days' incubation, but the cells degenerated rapidly. (In order to demonstrate viral hæmagglutinin it was necessary to absorb the serum and embryo extract with kaolin[7] to remove inhibitors of viral hæmagglutination.) Rapid cell degeneration occurred in similar cultures without virus and seemed to be due to the fact that the cells metabolized very actively, with rapid cell division, the new cells being detached from the glass. This behaviour was quite different from that of the cells kept in maintenance medium and it raised the possibility that the lengthy resistance to viral infection induced by a single dose of interferon might be due to the fact that the cells kept in maintenance medium were unable to divide.

In order to test this possibility cells treated with interferon were kept in maintenance medium and infected with West Nile virus, and after 3, 4 or 5 days'

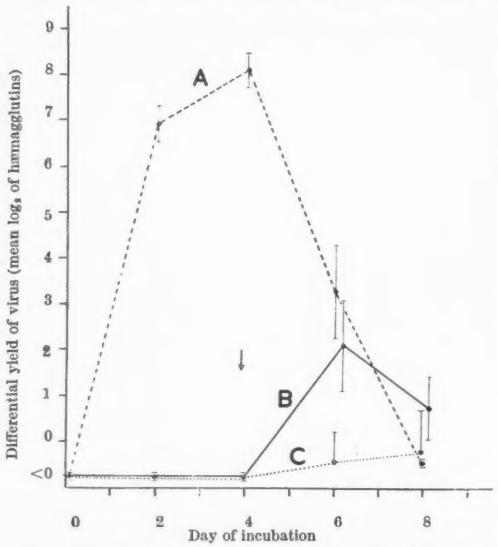

Fig. 1. Groups of 6 tubes each containing 5×10^6 chick fibroblasts in a simple maintenance medium were infected with $3·2\times10^5$ plaque forming units of West Nile virus. The medium was changed every second day and titrated for viral hæmagglutinin. The curves show the geometric mean hæmagglutinin titres and standard deviations of the virus yields for each two-day period. (Negative tubes were given an arbitrary score of −1 to facilitate calculations.)
Curve *A*, virus alone; curves *B* and *C*, cells were treated with interferon before virus was added. On the fourth day following addition of virus the medium was enriched with 5 per cent calf serum + 1·5 per cent chick embryo extract (curve *B*) and 5 per cent calf serum + 1·5 per cent chick embryo extract + interferon (curve *C*).

incubation, serum and chick embryo extract were added to stimulate cell division. The cultures at once showed active metabolism and in one experiment a distinct fall in *p*H was noted within 75 min. of adding the enriched medium. However, the resistance to viral infection broke down partially, as shown by the appearance of viral hæmagglutinin in the medium (Fig. 1, curve *B*, shows the type of result found). The most likely explanation of these findings is that in cells which are unable to divide, sufficient interferon to prevent virus multiplication is retained for a long period of time within the cells. But when cell division occurs, the interferon which is unable to replicate[8], is diluted until the concentration within cells is lower than that required to inhibit virus multiplication. In support of this hypothesis, the resistance of cells could be largely maintained by incorporating further interferon along with the serum and embryo extract each time the medium was changed (Fig. 1, curve *C*).

These experiments provide a model system which may be helpful in planning experiments on the protective effect of interferon in virus infections in animals.

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Glycosidases in the Mammalian Alimentary Tract

EPITHELIAL mucous secretions in mammals are comprised mainly of mucosubstances[1] consisting of some or all of the carbohydrates, glucosamine, galactosamine, galactose, fucose and sialic acid bound to protein[2]. In some secretions there are also small amounts of acidic aminopolysaccharides containing hexosamine, uronic acid and sometimes ester sulphate. Mucosubstances with the above composition, but excluding sialic acid, resemble blood-group substances, and fractions with high blood-group activity have been obtained from various secretions, including gastric juice, as well as from gastric mucosa.

In view of the prevalence of mucosubstances in the alimentary tract, and the widespread occurrence of the four glycosidases α-mannosidase, β-galactosidase, β-*N*-acetylglucosaminidase and β-glucuronidase in animal tissues[3], an investigation has been made of the distribution of these enzymes throughout the alimentary tract of several mammalian species, the wall itself and the contents of the lumen each being examined. Values for some sections of the alimentary tract and their contents, together with figures for pancreas and parotid gland, are given in Table 1. The same assay methods as before were employed[3], but the concentration of *p*-nitrophenyl α-mannoside used was 6 m*M*. Generally, figures for the alimentary tract tissue were fairly constant for each species throughout the length of the tract. For purposes of comparison, some values for liver and kidney are included; in some cases these or similar figures have already been published. A study of β-glucuronidase in alimentary tract contents has already been made[4]. It was also observed that in rats after a period of starvation (24 hr.), or several hours after ether administration, there was a definite, though somewhat variable, tendency for the glycosidase activities of the alimentary tract contents to rise. This may have been due to mechanical factors, rather than increased enzyme secretion.

While β-galactosidase may also have lactase activity, the presence of the other glycosidases in tissues known to secrete mucosubstances, together with the absence of any simpler glycoside molecule, suggests that such mucosubstances may well be natural substrates for this group of enzymes. Although not detected in the alimentary tract, mannose is a frequent component of mucoproteins from other sources.

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Table 1. GLYCOSIDASE ACTIVITIES IN THE ALIMENTARY TRACT, PANCREAS AND PAROTID GLAND OF VARIOUS SPECIES

Results are expressed as μgm. *o*-nitrophenol (β-galactosidase), *p*-nitrophenol (α-mannosidase and β-*N*-acetylglucosaminidase) or phenolphthalein (β-glucuronidase) liberated per gm. moist tissue in 1 hr. at 37° C. from the appropriate glycoside.

	α-Mannosidase					β-Galactosidase				
	Sheep	Ox	Pig	Rat	Rabbit	Sheep	Ox	Pig	Rat	Rabbit
Stomach (abomasum)	4,920		3,900	3,995	813	371		4,870	2,270	750
Stomach contents	347		282	129	26	139		212	145	49
Ileum	3,650	6,780	1,669	6,228	4,370	672	1,310	2,830	3,051	5,450
Ileum contents	523	5,930	2,451	166	2,140	0	3,260	7,070	100	2,041
Colon	2,210	5,460		4,470	1,068	654	1,090		4,130	2,095
Colon contents	693	675		945	110	80	207		234	0
Pancreas	2,100	1,416			517	428	486		1,150	268
Parotid	2,630	1,224				2,090	208			
Liver			2,955		4,190			4,530	4,710	1,042
Kidney			4,560		3,040			16,800	11,400	4,460

	β-*N*-Acetylglucosaminidase					β-Glucuronidase				
	Sheep	Ox	Pig	Rat	Rabbit	Sheep	Ox	Pig	Rat	Rabbit
Stomach (abomasum)			21,550	23,725	11,030	1,095		50		35
Stomach contents			1,812	293	139	4		52		4
Ileum	33,850	42,200	42,100	35,213	62,300	782	1,057	31	2,810	769
Ileum contents	1,890	40,500	43,500	439	27,230	110	860	80	260	250
Colon	19,920	36,000			88,000				3,450	276
Colon contents	4,290	1,775			2,060				2,955	1,448
Pancreas	8,100	6,190		4,830	12,980	240	52		860	105
Parotid	18,050					2,210	316			
Liver			69,700	44,610	36,600	7,210		220	19,340	6,220
Kidney			206,800	126,650	56,300			22	3,470	281

Isolation of Echinochrome *A* from the Spines of the Sea Urchin, *Diadema setosum* (Leske)

The naphthoquinone biochromes in the animal kingdom are only found in the group of sea urchins: the various colours (green, red, violet or black) of the spines and the tests of sea urchins arise from the calcium salts of these naphthoquinone pigments [1,2].

Echinochrome *A* [3,4] (7-ethyl-2, 3, 5, 6, 8,—pentahydroxy–1, 4–naphthoquinone) which was recognized as the naphthoquinone pigment in the ovaries of the sea urchin *Arbacia lixula* (Linn.), has been found in the tests and the spines of the four species of sea urchins: *Strongylocentrotus purpuratus* (Stimpson)[5], *Paracentrotus lividus* (Lam.)[1,3], *Echinus esculentus* (Linn.)[6] and *Echinarachnius mirabilis* (Ag.)[7]. Recently, a naphthoquinone pigment isolated from the dark violet-black spines of the sea urchin, *Diadema setosum* (Leske) (Japanese name, 'gan gaze uni') was identified with echinochrome *A*.

Spines washed with water were dissolved in dilute hydrochloric acid and the pigment was extracted therefrom with ether and transferred into saturated sodium bicarbonate solution. The pigment was extracted again in ether, after acidification with dilute hydrochloric acid, and purified by column chromatography on calcium carbonate and recrystallization from dioxane-water. About 6 mgm. of the pure material were obtained from each 100 gm. of the spines. The pigment forms dark red-brown needles, m.p. 214°–215° and shows absorption maxima at 255, 340, 467, 490, 527 mμ in chloroform solution. Ferric chloride reaction gives a dirty black-violet colour and a violet precipitate appears when it reacts with methanolic lead acetate. The percentage of C and H was 53·94 and 3·90 respectively (calc. for $C_{12}H_{10}O_7$: C, 54·14; H, 3·79). The trimethyl-derivative was obtained by methylation with diazomethane in ethereal solution, as long red needles which were crystallized from dioxane-water. It melts at 130°, is not soluble in sodium bicarbonate solution, but dissolves in dilute sodium hydroxide with a blue colour. Absorption maxima were at 323, 476, 502, 537 mμ in chloroform solution. The percentage of C and H was 58·85 and 5·17 respectively (calc. for $C_{12}H_7O_4(O{\cdot}CH_3)_3$: C, 58·35; H, 5·29). Treatment with zinc dust, pyridine and acetic anhydride gave the leucoacetyl derivative as colourless fine rods, m.p. 240° (decomp.). The absorption maximum was at 295 mμ in methanol solution. The dehydro-derivative, formed by treatment with silver oxide, showed absorption maxima at 260, 319, 392 mμ in methanol solution.

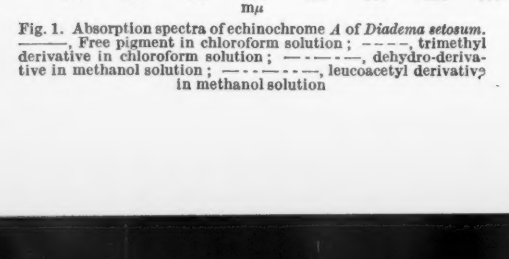

Fig. 1. Absorption spectra of echinochrome *A* of *Diadema setosum*. ———, Free pigment in chloroform solution; – – – –, trimethyl derivative in chloroform solution; — — — —, dehydro-derivative in methanol solution; — · — · — · —, leucoacetyl derivative in methanol solution

Mixed melting point determinations have been carried out with echinochrome *A*, trimethylechinochrome *A* and leucoacetylechinochrome *A* (isolated from *E. mirabilis*)[7], and in each case no depression of the mixed m.p. was observed. The infra-red spectrum of this pigment and echinochrome *A*[7] was also fairly agreeable. Full details of this work will be published elsewhere.

I wish to express my gratitude to Prof. Y. Nakamura and Prof. T. Satto, of Hokkaido University for their encouragement and guidance. I am also grateful to Prof. R. Kamohara and Prof. T. Yatuzuka of Kochi University for offering me every possible assistance in collecting samples and to Dr. M. Inoue of Takeda Research Laboratory for the elemental and infra-red analysis.

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Occurrence of 4-Hydroxypipecolic Acid in *Acacia* Species

Extraction of *Acacia excelsa* heartwood gave an imino-acid (0·2 per cent), m.p. 294° (decomp.), $[\alpha]_D^{20}$—13·4° (1 per cent in water), characterized by an *N*-benzoyl derivative, m.p. 172°, and identified as *trans*-4-hydroxypipecolic acid. The acid was later isolated from the wood of other *Acacia* species, and was more conveniently obtained from the fresh leaves of *A. oswaldii* (0·25 per cent yield). The imino-acid fraction, isolated by means of the *N*-nitroso derivatives[1], sted alnsicomost entirely of proline, pipecolic acid, and the hydroxypipecolic acid, which crystallized readily from aqueous ethanol. The naturally occurring *trans*-isomer was epimerized by aqueous barium hydroxide (155°, 12 hr.) to a mixture of *cis*- and *trans*-4-hydroxypipecolic acid, and on paper chromatograms developed with butanol-acetic acid-water (4:1:5) the *cis*-acid (R_F 0·17) was indistinguishable from an authentic specimen, but was clearly separated from *cis*-3-hydroxypipecolic acid (R_F 0·24). A further distinction between the 3- and 4-hydroxypipecolic acids is that the former acid is decomposed when heated with alkali under conditions that cause epimerization of 4-hydroxypipecolic acid, and 3-hydroxypipecolic acid therefore resembles other β-hydroxy-α-amino-acids in its alkali-lability[2]. The naturally occurring *trans*-4-hydroxypipecolic acid ran in butanol-acetic acid-water with the same R_F(0·21) as 5-hydroxypipecolic acid from dates[1], but the two acids were separated on paper chromatograms developed with water-saturated phenol, and the 4-hydroxypipecolic acids were also distinguished by giving with ninhydrin a characteristic grey colour which showed deep red fluorescence under ultra-violet light.

Isolation of 4-hydroxypipecolic acid was first reported by Virtanen and Kari[3], and the same acid was isolated from *Armeria maritima* by Fowden[4] who tentatively revised its structure to 3-hydroxypipecolic acid. It now appears that 4-hydroxypipecolic acid is the true structure of the acid isolated by Virtanen and Kari and by Fowden as a

sample provided by Dr. Fowden proved chromatographically indistinguishable from our *trans*-4-hydroxypipecolic acid, and its was similarly epimerized by hot baryta. Structural and stereochemical investigation of the *trans*-4-hydroxypipecolic acid from *Acacia* species is continuing, and details will be published elsewhere.

We thank Dr. L. Fowden for a sample of the acid from thrift and for comparing it with our imino-acid, and we are grateful to Dr. H. Plieninger and Dr. H. Vanderhaeghe respectively for samples of *cis*-3- and *cis*-4-hydroxypipecolic acids. This work was carried out during tenure of a General Motors Holden Fellowship (by P.I.M.).

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ANIMAL PHYSIOLOGY

Increase by Chlorothiazide of the Paralysing Activity of *d*-Tubocurarine Chloride

It is a well-established fact that chlorothiazide potentiates the hypotensive effects of ganglion-blocking agents[1-2]. However, the way this potentiation is brought about is not clear. It has been thought that chlorothiazide acts either by a direct hypotensive action[2], or by sodium depletion[3], or by reduction in plasma-volume[3], or as in the case of mecamilamine and possibly of pempidine, by a reduction in renal excretion[4] of the ganglion-blocking agents. From a pharmacological view-point there is a good deal of similarity between the neuromuscular junction and the gangliarsynapsis.

We have therefore investigated whether the paralysing activity in a rabbit, treated with *d*-tubocurarine chloride, could be modified by a previous intravenous injection of chlorothiazide.

In evaluating the paralysing activity of *d*-tubocurarine we have taken into account: (*a*) the appearance of muscular insufficiency that allows the animal, when set in a lateral position, quickly to resume its normal stand-up position (partial paralysis); (*b*) the appearance of a muscular insufficiency that deprives the animal of its ability to resume its stand-up position (total paralysis); (*c*) the animal's death owing to a respiratory insufficiency.

We have summarized our results in Table 1.

It is evident that chlorothiazide pretreatment potentiates the neuromuscular-blocking activity of *d*-tubocurarine. Hidrochlorothiazide, on the other hand, is ineffective in 10–100 mgm./kgm. dose intravenously in increasing *d*-tubocurarine paralysis.

The mechanism of chlorothiazide action is not clear as yet.

Our results will be published elsewhere in detail.

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Are Mucosal Nerve Fibres Essential for the Peristaltic Reflex?

Recently Bülbring and co-workers[1,2] abolished the peristaltic reflex in an isolated piece of intestine by scraping off its mucous membrane and assigned an essential role in the initiation of the reflex to processes of sensory neurones which are distributed to the intestinal mucosa.

In the experiments reported here, an attempt was made to destroy the mucous membrane selectively by local administration of a protein-precipitating chemical. Silver nitrate and tannic acid were chosen as suitable chemical agents.

The method of eliciting the peristaltic reflex in an excised loop of guinea-pig ileum mounted in an organ bath[3], was modified so that the output of each peristaltic wave could be directly measured; this permitted to distinguish unequivocally between peristalsis which propelled fluid in a cephalocaudal direction, and pendular activity which did not. In order to avoid formation of silver chloride, the tubings and the intestinal lumen were thoroughly flushed with distilled water before and after the administration of silver nitrate.

Among various concentrations tried, a 30 per cent silver nitrate and a 20 per cent tannic acid solution proved suitable when left in contact with the mucosal surface for about 10 and 30 sec. respectively. After such treatment peristaltic activity continued in its normal pattern of co-ordinated contractions of the longitudinal and circular muscle layers. The amount of fluid expelled was generally slightly reduced and so was the size of the longitudinal contractions and at the same time the response of the longitudinal muscle to acetylcholine, indicating that some damage had occurred to all layers of the intestinal wall. As controls revealed, part of this could be accounted for by the mechanical strain exerted on the wall by forcing the solutions and wash fluid through the lumen in a specified time. However, in two experiments, peristaltic activity was even increased after treatment with 30 per cent silver nitrate solution.

Histological investigation of these preparations, carried out by Dr. M. R. Crompton of the Department of Histology, showed that most of the mucous membrane and parts of the muscularis mucosae were destroyed.

Table 1. Paralysing activity of *d*-tubocurarine chloride in rabbits treated with chlorothiazide and hydrochlorothiazide

Pretreatment	mgm./kgm. i.v.	*d*-tubocurarine μgm./kgm. i.v.	Interval between the two treatments min.	Animals with partial paralysis/ treated animals	Animals with total paralysis/ treated animals	Dead animals/ treated animals
—	—	125	—	11/18	0/18	0/18
Chlorothiazide	100	125	10	7/7	7/7	2/7
"	"	125	15	2/2	2/2	0/2
"	"	125	30	3/3	0/3	0/3
Hydrochlorothiazide	100	125	1–14	7/9	1/9	0/9
"	10	125	6–13	4/6	2/6	0/6

There was only little variation between different preparations and different sections of the same preparation. The demarcation line between necrotic and normal tissue lay in the neighbourhood of ganglion cells of Meissner's plexus and was particularly conspicuous in preparations treated with silver nitrate where deposits of free silver developed if the preparation was exposed to light during fixation. Thus the damage reached approximately the same depth of the intestinal wall as in the experiments of Bülbring *et al.* after the mechanical removal of the mucosa.

The findings therefore permit the conclusion that the mucosa and the nerve fibres situated there, do not play an indispensable role in the peristaltic reflex of the guinea pig ileum.

A detailed description of these findings and the methods used will be published elsewhere.

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Extrahepatic Metabolism of Ethanol in Man

It is generally assumed that only small amounts of ethanol are metabolized outside the liver in man[1,2]. The results obtained when working at very low concentrations of ethanol, where the metabolic capacity of the liver is no longer fully saturated, are not consistent with this view. When the concentration of ethanol in the blood reaching the liver is below 50–60 mgm./l., the concentration in the liver vein, obtained by catheterization, has been found to be zero. If extrahepatic metabolism can be excluded the amount metabolized at these levels of ethanol must be proportional to the liver blood flow and to the concentration in the blood. Measurements of the liver blood flow by means of ethanol and bromsulphalein have shown, that the blood flow during experiments at periods similar to those mentioned below, is almost constant[3,4]. The amounts of ethanol metabolized can therefore be proportional only to the concentration in the blood.

If ethanol is infused intravenously at a constant rate the concentration in the blood will be constant after 60 minutes and the amount metabolized will then be identical to the amount infused. The metabolism of ethanol has been investigated in 10 apparently healthy students by this technique and the rate of infusion was changed two to three times during the same experiment. If the ethanol was only metabolized in the liver a strictly proportionality was to be expected between the amount infused and the concentration in the blood. By plotting the results in a graph with ethanol metabolized per minute for the abscissa and the concentration in the blood for the ordinate, a straight line passing through the origin should be obtained. As will be seen from Fig. 1, a straight line was obtained, but in all cases it passed to the right of the origin.

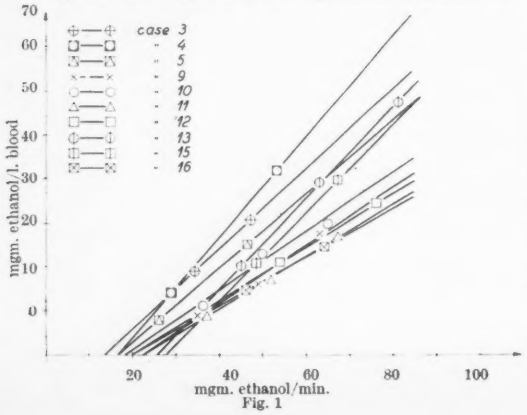

Fig. 1

Table 1

Case	mgm. ethanol oxidized per min.	mgm. ethanol/l. blood	Liver blood flow (ml./min.)	Ethanol oxidized extra-hepatically mgm./min.)
3	34	19	1770	14
	47	31	1520	
4	29	14	2070	17
	53	42	1260	
5	26	8	3250	17
	46	25	1840	
12	54	21	2570	20
	76	34	2240	
15	48	21	2290	27
	67	40	1680	
16	46	15	3070	20
	64	25	2560	
9	35	9	3890	23
	49	16	3060	
	63	28	2250	
10	36	11	3270	18
	50	23	2170	
	65	30	2170	
11	37	9	4110	23
	52	17	3060	
	67	27	2480	
13	45	20	2250	26
	63	40	1575	
	81	58	1400	

The best explanation for this result seems to be extrahepatic metabolism of ethanol. The point where the line crosses the abscissa indicates the amount of ethanol metabolized outside the liver with a mean value (Table 1) from 10 experiments of 20·5 mgm. per minute (standard deviation 4·2). The constancy of the extrahepatic metabolism down to very low concentrations in the blood seems to indicate an organ, or organs, with a low concentration of alcohol-dehydrogenase and a high blood flow, but no information is at present available on this location.

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Natriferin: A New Hormonal Principle in the Neurohypophysis of Certain Vertebrates

The active transport of sodium is an important function of the amphibian skin; this transport can be increased by extracts of mammalian neurohypophysis[1]. In *Rana esculenta*, oxytocin alone is active, vasopressin having only a small effect corresponding to its intrinsic oxytocic activity. On the other hand, neurohypophyseal extracts of Amphibia and fish influence this transport at such low concentrations, that it would seem reasonable to postulate that their action is due to an unknown principle, more specific to sodium transport than is oxytocin itself[2,3].

Acetic extracts were prepared from acetonic powders of entire hypophysis or neurohypophysis from various aquatic vertebrates (elasmobranchs, marine and freshwater teleosts, amphibians) and from mammals. The oxytocic activity of these extracts was

measured by their effects on rat uterus contractions[4] and their 'natriferic' (that is, sodium transporting) activity, by their effects on the net flux of sodium produced by *Rana esculenta* skins, as measured by the 'short-circuited current' (Ussing's technique modified by Morel *et al.*[2]). As a standard of reference for both these biological activities, synthetic oxytocin ('Syntocinon', Sandoz) was used, permitting the activities to be expressed quantitatively in terms of mU/mgm. dry powder.

Table 1. Oxytocic and Natriferic Activities of the Entire Hypophysis (H) or Neurohypophysis (N) in Different Vertebrates

Species	Oxytocic activity* (± S.E.)	Natriferic activity* (± S.E.)
Hexanchus griseus (H) ..	2·0 ± 0·25	4·6 ± 0·4
Scyliorhinus canicula (H)	6·8 ± 1·0	15·1 ± 1·8
Scomber scombrus (H) ..	450 ± 33	4980 ± 720
Germo alalunga (H) ..	595 ± 45	5610 ± 1352
Blennius gattorugine (H)	108 ± 11	1030 ± 138
Trutta trutta (H)	45·5 ± 6·0	443 ± 116
Rana esculenta (N) ..	1066 ± 170	12800 ± 1130
Bufo bufo (N)	1000 ± 16	12050 ± 1165
Rat (N)	1950 ± 46	1990 ± 47
Ox (N)	909 ± 63	1356 ± 75
Pig (N)	1460 ± 117	1610 ± 90

*Both activities expressed in mU oxytocin/mgm. dry powder.

Table 1 shows the results of this investigation: the elasmobranch extracts have a very low biological activity, the mammalian extracts, a natriferic activity more or less equal to their oxytocic activity, whereas the teleostean and amphibian extracts exhibit a far greater natriferic than oxytocic activity. Fig. 1 represents these results in terms of the ratio of natriferic to oxytocic activity in the various species studied. This ratio is approximately 1 in the mammals, indicating that oxytocin alone is the active principle in both tests. The teleosteans and amphibians show ratios of roughly 10. In other words, their oxytocic activity cannot account for their natriferic activity. Further experiments were performed to test whether the discrepancy between the two activities could in fact be due to antehypophyseal hormones, intermedin or vasopressins. All these substances were found to be devoid of natriferic activity. Furthermore, the oxytocic and natriferic activities of the hypothalamus of *Rana esculenta* were measured and found to be 5·0 ± 0·4 mU and 52·7 ± 4·5 mU respectively, giving a ratio of 10·5, comparable with that found for the neurohypophysis. The above experiments thus indicate the existence of a new factor in amphibians and teleosts, probably of hypothalamic origin, and responsible for the natriferic activity. The term 'natriferin' has been proposed to designate this principle[3,5].

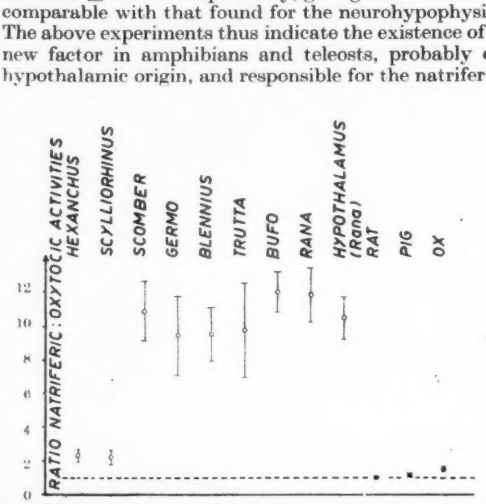

Fig. 1. The ratio natriferic : oxytocic activities (±S.E.) in different vertebrates

There is evidence to support the hormonal nature of natriferin. Thus it acts *in vitro* at very low concentrations (1/10,000 of a neurohypophysis of *Bufo*, that is, 0·0125 μgm. of dry powder per ml.). Neurohypophyseal extracts injected *in vivo*, into normal individuals of *Bufo* and *Rana* (that is, kept in tap water) have also a far greater effect on the active sodium uptake than would be expected from their actual oxytocic contents. Furthermore, in individuals of *Rana esculenta* adapted to a high salinity in the external medium, there is a diminution of active transport of sodium by the skin and a corresponding reduction in the oxytocic and natriferic activities of the neurohypophysis, whereas the antidiuretic activity of the gland remains unchanged[3,6].

The physiological role of natriferin would seem to lie in relation to osmoregulation. Such a function is indicated by its specificity of action on the active transport of sodium and also by its ecological distribution within the aquatic vertebrates. Its absence in elasmobranchs may be explained by their very special solution of osmoregulatory problems[7]. One fact should be stressed: the ratio between natriferic and oxytocic activities remains constant throughout the amphibian and teleostean species studied, despite considerable variation in absolute concentration of active principle in the glands. This points in favour of the hypothesis that a single substance, common to all these animals, is responsible for both natriferic and oxytocic activities. Natriferin, in fact, would appear to be a substance closely related to oxytocin. Its relationship with the 'water-balance principle'[8] remains to be studied.

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Vagal Afferents in the Monkey

Vagal afferents in several vertebrate species have been extensively described[1], but we are unaware of any previous recording of afferent activity in the vagus nerve of a primate. The opportunity arose to study these in the monkey *Macaca mulatta*.

In the supine animal, anæsthetized with 'Nembutal', the left cervical vagus was exposed. Under paraffin oil the sheath was opened and individual fine strands of nerve were sectioned, separated peripherally and subdivided and the resulting filaments were laid across a pair of silver recording electrodes. The electrical activity in such filaments was displayed with one beam of a dual beam oscilloscope. The electrocardiogram and usually arterial, central venous and intrapleural pressures, registered with strain gauge manometers, were displayed with the second beam by the use of a multichannel beam splitter. Photo-

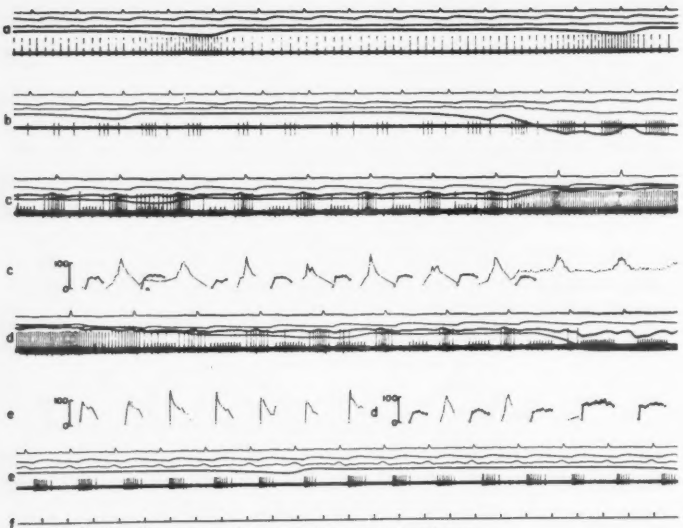

Fig. 1. Each oscilloscope record, top to bottom: electrocardiogram, aortic pressure, central venous pressure, intrapleural pressure (inspiration downwards), afferent vagal impulses. Graphs: impulse frequency, per sec., on same time-scale as oscilloscope records. *a*, discharge characteristic of continuously firing pulmonary stretch receptor during two normal respirations; *b*, discharge characteristic of single atrial stretch receptor during normal respiration and with application of negative pressure to the trachea; *c*, largest spikes, discharge characteristic of pulmonary stretch receptor with pronounced cardiac rhythm persisting during application of positive pressure to trachea. Also prominent is the discharge of an atrial stretch receptor. Discharge frequencies for the two receptors noted are plotted below; *d*, shortly after record *c*, comparing effects on the same two receptors of applications of positive, then negative, intratracheal pressure; discharge frequencies during the latter manœuvre plotted below; *e*, discharge characteristic of arterial baroreceptor, with frequency plotted above; *f*, 60 c.p.s. time marker and 0·2-sec. lines

graphic records were obtained using moving paper. Nerve impulse frequencies were plotted from the records using a miniature direct-plotting nomogram[2].

In six monkeys, afferent discharges were recorded from about forty individual receptors. The temporal features of their discharge patterns and their responses to respiratory manœuvres were generally characteristic of types of receptors described in other mammals. Easily recognized were the discharges of pulmonary stretch receptors of several kinds, arterial baroreceptors and cardiac atrial receptors. No attempt was made to identify the exact site of the receptors by manipulation of the viscera. Two types of pulmonary stretch receptor behaviour were commonly encountered; the first (Fig. 1*a*) showed a continuous discharge with the usual respiratory modulation, while the second (Fig. 1 *c*, *d*) possessed an adventitious cardiac rhythm. The prominence of the latter type of discharge, although observed in experimental circumstances, leads one to speculate that the cardiac component, usually associated with systole, may represent physiologically significant information. In nine atrial receptors, the main pattern of discharge was diastolic in timing (Fig. 1 *b*, *c*, *d*), indicating their sensitivity to distension but not to pressure; one atrial receptor only was encountered with a pronounced atrial systolic discharge, but with a diastolic component as well. Arterial baroreceptor fibres were found in four of the monkeys (Fig. 1 *e*) and unlike the atrial receptor fibres, these appeared to travel together in a discrete region within the main trunk of the vagus, as they do in the dog.

The demonstration in the monkey of the usual vagal afferents was not unexpected. Of special interest, however, in view of their proposed role as thoracic blood volume receptors[3,4], is the occurrence of atrial stretch receptors in a primate which, like man, spends much of its time in the upright position.

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Prevention of Foetal Development by Enzyme Inhibition

It has been shown recently that fœtuses of rats and humans at a definite phase of development produce histamine at a very high rate.[1-3]. Towards the end of pregnancy an average sized set of rat fœtuses produces about ten times more histamine than the mother in a given time and about fifty times more per unit weight. The fœtal histamine enters the mother's circulation and is then in part destroyed, in part excreted in the urine. Fœtal tissues bind histamine only loosely, they thus contain little, and produce it at a high rate[3]. As a working hypothesis it would seem that the striking histamine-forming capacity of the fœtus might be related to growth in general. To test this hypothesis we have investigated histamine formation in the regenerating liver.

In the rat, the median and left lateral lobes of the liver, amounting to about two-thirds of the whole, can be removed without difficulty[4]. The remnant grows and reaches the size of the normal liver within about 20 days. In partially hepatectomized rats it was found that the rate of histamine formation, as reflected in the urinary excretion of the amine, is considerably increased during liver regeneration. Also, when radioactive histidine was injected and histamine formation followed, it was seen that decarboxylation took place at a higher rate during liver regeneration than before the operation. In the fœtus, as in the regenerating liver, rapid histamine formation is not due to mast cells since such cells have only been found in fœtal skin where the histamine-forming capacity is rather low.

These observations encouraged us to study the effect of inhibition of histamine formation on fœtal development. First, we had to discover a suitable way of producing this inhibition. To this end we made use of two new methods for the determination of the rate of endogenous histamine formation. One depends on the fact that in the female rat fed on a specially compounded histamine-free diet, the amount of free histamine excreted in the urine parallels the amount of endogenously formed histamine[5]. The other method, introduced by Schayer, measures the amount of radioactive histamine formed and excreted

following subcutaneous injection of radioactive histidine. Because a specific inhibitor of histidine decarboxylase has not yet been found, we used semicarbazide, the least toxic among known inhibitors of amino-acid decarboxylases. On omitting the coenzyme of histidine decarboxylase, pyridoxine, from the diet, semicarbazide in fairly small doses inhibited the rate of histamine formation by as much as 85 per cent. Under this treatment the rats fared reasonably well as regards appetite, exercise and maintenance of body weight.

In the rat the effect on fœtal development of enzyme inhibition due to semicarbazide superimposed on a pyridoxine-deficient diet has been studied. Procedures and doses were such as to reduce the rate of histamine formation to about 15 per cent of normal. In one group of rats fed on the pyridoxine-deficient diet, semicarbazide was given for eight days from the seventh day of pregnancy onwards, that is from about the day of implantation of the ovum. At the nineteenth day, when the weight of the normal fœtus is about 2 gm., there were only tiny remnants of fœtuses in the form of plaques of disintegrated material, whilst the placentæ were small and appeared to be in a state of regression (Fig. 1). In another group fed on the pyridoxine-deficient diet, semicarbazide was given for eight days from the fifteenth day of pregnancy. On killing the animals at the 22nd day the findings were largely the same as in the other group. The fœtuses were dead and mummified and their growth appeared to have been arrested at about the seventeenth day, that is the day when enzyme inhibition became maximal. In controls fed for the same period of time on the pyridoxine-deficient diet, or simply injected with semicarbazide, no abnormalities in the course of pregnancy and fœtal development were noted.

We have not yet investigated to what extent other enzymes besides histidine decarboxylase are inhibited by the measures which in these experiments prevent fœtal development. Inhibition of histamine formation appears to be involved for the following reasons. Of the substances the formation of which is known to be inhibited by semicarbazide, only histamine has so far been found to be specifically related to fœtal development. It has been shown by G. B. West, as well as by our colleague H. Westling, that the formation of 5-hydroxytryptamine is not increased in rat pregnancy (personal communications). Further, semicarbazide in doses larger than the ones used in our experiments did not inhibit the endogenous formation of 5-hydroxytryptamine in the guinea pig[6]. Diamine oxidase (histaminase) is known to be inhibited by semicarbazide. It has, however, been shown that complete inhibition of this enzyme by aminoguanidine has no detectable effect on the course of pregnancy or the fitness of the newborn[1]. Nevertheless, final proof of the dependence of fœtal development on a high rate of histamine formation will have to wait until a specific non-toxic inhibitor of histidine decarboxylase becomes available. The recent discovery of a rich source of this enzyme, fœtal rat liver[3], is likely to encourage the search for a specific inhibitor.

Fig. 1. The uterine contents of two rats at the nineteenth day of pregnancy. Left, undisturbed pregnancy; right, after enzyme inhibition. The latter animal was fed a pyridoxine-deficient diet from the first day of pregnancy onwards, semicarbazide was injected between the seventh and fifteenth days starting with 50 mgm./kgm. twice daily on the seventh day, followed by 75 mgm./kgm. twice daily. On discontinuing semicarbazide a normal diet was given.

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Effect of Sexual Maturation and Castration on the Sex Chromatin Pattern in the Male Rat

The 'nucleolar satellite' described by Barr and Bertram[1] is generally considered to be of chromosomal origin and is, therefore, determined genetically. That is why, theoretically, the incidence of sex chromatin bodies is not influenced by age, or exogenous and endogenous factors. Davidson and Smith[2], recognizing the sexual dimorphism of leukocytes, thought that the 'drumstick'-form, which is characteristic of females, represented a fusion of the heterochromatic segments of two paired *X* chromosomes. However, certain signs would seem to indicate that the chromatin condensations in leukocytes do not show, in every respect, the properties of the 'nucleolar satellites'. In addition to the female 'drumstick', several authors[3,4] have observed so-called pseudoforms, the occurrence of which is also related to actual sex. At the same time it cannot be unambiguously explained how it is possible that chromosome pairs have so many, variegated and yet nonspecific morphological equivalents. The foregoing facts and the presumptive relationship between sexual hormones and leukocytes has tempted us to study in leukocytes the hormonal relations of the sex chromatins, especially those of the pseudo-forms.

In this work Kosenow's method was applied, which uses the equation $Q = A + B/C$, where A represents 'drumsticks', and B and C pseudoforms. Thus the numerical changes in the different types are made conspicuous.

64 male albino rats of a Wistar strain and of known age were divided into four groups of 16. Blood-smears were taken weekly, the slides fixed with methanol and stained with a Giemsa-solution. 500 neutrophil leukocytes were counted, and the quotient Q, which in the mature male rats was less than 0·6, determined.

In the first group of animals counts were made from the 2nd to the 12th week of life (Fig. 1).

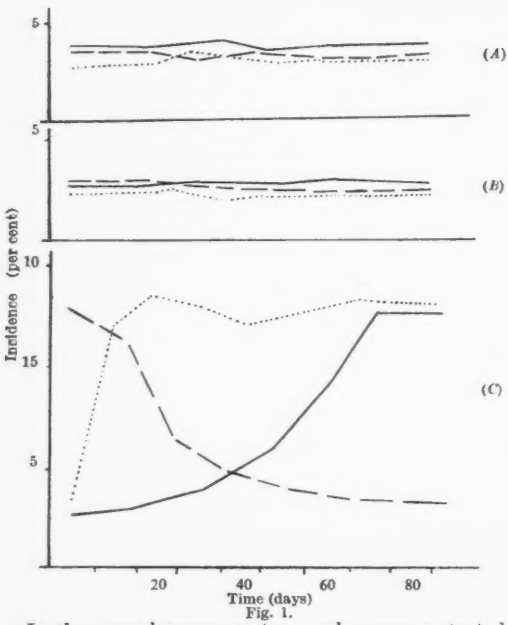

Fig. 1.

In the second group, mature males were castrated and observed for 10 weeks.

The two groups, consistently and regardless of age, showed the same values for forms *A* and *B* (3·5 and 2·5 per cent on an average). At the same time, there was a considerable difference in the incidence of form *C*; its frequency, about 2–3 per cent at birth, gradually increased with age (full line), reaching about 12 per cent at sexual maturity, which is characteristic of mature males. On the other hand, after castration form *C* decreased in number (dashed line) and, within a month, approached the percentage for the newly born male rats. These results suggest that the incidence of form *C* is in relation with the actual androgen-level. To elucidate the question, a new experiment was carried out with a third group of animals.

A dose of 1 mgm./day (altogether 36 mgm.) of a testosteron-propionat ('Androfort', Kőbányai Gyógyszerárugyár, Budapest) was injected into young male rats of 3 weeks of age. As early as by the 10th day sex chromatin form *C* was found to have reached a frequency characteristic of mature animals (dotted line), which had been expected to occur only six or eight weeks later, as seen in the first group under physiological conditions.

To exclude a possible aspecific steroid effect, 2 mgm. of an oily cholesterin suspension were administered to the fourth group, which, however, failed to influence the sex chromatin pattern.

It should be emphasized that, as has been reported by other workers[5,6], after administration of testosterone no changes whatever were seen in the sex chromatin pattern of the epithelial cells of skin.

These findings indicate that the incidence of form *C*—in contrast to *A* and *B*—is influenced by age and androgen. This has led to the conclusion that form *C* in neutrophil leukocytes should not be regarded as a real sex chromatin body. The incidence of form *C* being an important factor in Kosenow's formula, it would seem that his method for the determination of sex by blood smears cannot always be relied upon to demonstrate the real genotype in the rat. This view has been supported by our observations upon female animals, the results of which will be reported at a later date.

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A Peripheral Effect of the Bromide Ion on the Contraction of Striated Muscle

According to current literature, the main effects of the bromide ion are on the central nervous system. We have observed a hitherto undescribed but well-defined effect upon striated muscle in the following circumstances.

The diaphragm - phrenic nerve preparation in the rat described by Bülbring[1] was modified so as to use it for alternative supramaximal direct and indirect stimulation[2]. Upon replacement of normal Tyrode solution by Tyrode solution in which an equimolar concentration of sodium bromide was substituted for sodium chloride, both types of contractions were enhanced (see Fig. 1). The effect could be repeated

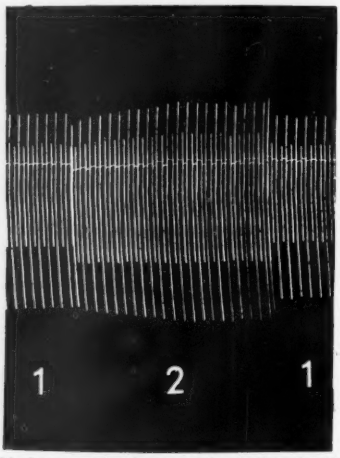

Fig. 1. Diaphragm – phrenic preparation in the rat with alternatively direct and indirect stimulation. 1, normal Tyrode solution; 2, Tyrode solution with sodium bromide.

several times on one and the same preparation. The bromide effect disappeared on continued exposure to the bromide Tyrode solution. It was also observed with preparations in which indirect excitability was completely inhibited by a high concentration of *d*-tubocurarine.

Experiments designed to analyse this phenomenon in more detail are in progress. They will be reported upon elsewhere.

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Sodium Chloride Intake and Urinary Histamine in Adrenalectomized Rats

It has been found by Schayer and his co-workers that adrenalectomy induces an increase in the rate of histamine formation in some tissues of the rat[1,2]. This increase may be the cause of the increased tissue histamine content[3–5]. Hicks and West pointed out that the tissue histamine content in adrenalectomized rats could be kept at a normal level by giving 0·9 per cent sodium chloride as drinking fluid[6]. A similar effect was observed by Rose and Browne[3]. During experiments on the effect of the adrenocortical hormones on the urinary excretion of histamine in normal and pregnant rats we made an observation which is of interest in this connexion.

White female rats were kept in metabolism cages and fed a dry cake diet *ad libitum*. The diet had a low histamine content (less than 0·8 μgm./gm.) and contained about 0·4 per cent sodium chloride and about 0·7 per cent potassium chloride. The rats were allowed to drink either distilled water or a 0·9 per cent sodium chloride solution *ad libitum*. Urine was collected in 24-hr. specimens and its histamine content estimated on the guinea pig ileum. In most cases the rats were given a daily subcutaneous injection of aminoguanidine sulphate (20 mgm./kgm.) to prevent destruction of histamine by histaminase (for example, ref. 7). The urinary excretion of histamine was followed before and after adrenalectomy and the following changes were observed:

(1) In animals drinking water there was no or a small increase after removing the adrenal glands.

(2) In animals drinking 0·9 per cent sodium chloride there was a progressive and distinct increase of the histamine excretion.

(3) In adrenalectomized rats kept on water for 6–8 days after the operation the substitution of sodium chloride for water as drinking fluid caused an immediate increase in urinary histamine (Fig. 1). The high urinary histamine of adrenalectomized rats kept on saline could be lowered by changing over to water (Fig. 1).

(4) Mock-adrenalectomized rats showed no significant changes in the levels of urinary histamine.

From these observations and those of the other workers it seems probable that there is an increased formation of histamine in the adrenalectomized rat regardless of whether it is allowed to drink water or 0·9 per cent sodium chloride. In animals kept on water the formed histamine accumulates in the tissues and little is excreted. In animals kept on sodium chloride the histamine does not accumulate to the same extent but is excreted in the urine.

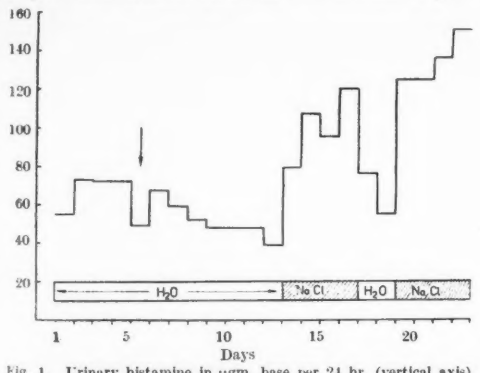

Fig. 1. Urinary histamine in μgm. base per 24 hr. (vertical axis) in female rat before and after adrenalectomy (at arrow). The drinking fluid was distilled water or 0·9 per cent sodium chloride as indicated. The rat was injected with 20 mgm./kgm. of aminoguanidine sulphate once daily under the skin.

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Effect of Sulphonamides on the Phagocytic Activity of the Reticulo-Endothelial System

The effect of various steroids, stilbenes, antibiotics and other substances on the phagocytic activity of the reticulo-endothelial system has been reported previously by Nicol and his co-workers[1,2,3]. It was shown that some of these substances stimulate the reticulo-endothelial phagocytes, some have little or no effect, and some are active depressants. So far, diethylstilbœstrol has been found to be the strongest stimulant and cortisone the most powerful depressant. Florey[4] has stated that it is possible that some chemotherapeutic agents depend for their complete effectiveness on the action of phagocytes. In this connexion it may be stated that our results with antibiotics[3] showed that these substances have little or no effect on the phagocytosis of particulate carbon.

The following work was designed to study the effect of sulphonamides on the phagocytic activity of the reticulo-endothelial system to find out whether or not these compounds depend for their effectiveness on the action of the reticulo-endothelial phagocytes.

The present experiments were carried out on 186 male white mice (*T.O.* Swiss strain) of 18–25 gm. body-weight. Thirteen sulphonamide compounds were investigated. The drugs were taken up in propylene glycol and the dose of sulphonamide given was 1 mgm. in 0·05 ml. propylene glycol once daily for 6 days. Twelve animals were used to investigate each compound, six receiving the drug orally and the other six subcutaneously. On the 8th day after the commencement of sulphonamide treatment the phagocytic activity was assessed by the rate of disappearance of a known amount of carbon from the circulating blood[5], the procedure used being that described in previous communications[3].

Thirty of the animals were used as controls. They were given 0·05 ml. propylene glycol once daily for 6 days and on the 8th day the phagocytic activity was assessed by the carbon method[3]. Half of the control animals received the propylene glycol orally, the other half subcutaneously.

The results are shown in Table 1. The control animals showed an average phagocytic index or K value of $16 \pm 2{\cdot}4$ after oral administration of propylene glycol and $18 \pm 4{\cdot}4$ after subcutaneous administration. Compared with the control values, it can be seen that the sulphonomides used, with the exception of sulphadiazine, have little or no effect on the phagocytic activity of the reticulo-endothelial system, and there is no significant difference between the results

Table 1. Effect of Various Sulphonamides on the Phagocytic Activity of the Reticulo-Endothelial System.

Sulphonamide used	Phagocytic Index (K value) after oral administration	Phagocytic Index (K value) after subcutaneous administration
Sulphaguanidine	20 ± 6·3	15 ± 3·1
Sulphamethoxypyridazine	19 ± 4·5	13 ± 2·2
Sulphamerazine	18 ± 3·7	13 ± 3·6
Acetazolamide	18 ± 2·6	15 ± 4·9
Sulphanilamide	16 ± 5·2	9 ± 4·5
Sulphadimidine	15 ± 2·6	16 ± 2·2
Sulphathiazole	15 ± 4·8	13 ± 3·0
Phthalylsulphathiazole	15 ± 1·4	15 ± 3·3
Succinyl sulphathiazole	14 ± 4·6	15 ± 3·3
6-sulphonilamido-2-4-dimethyl pyrimidine	13 ± 1·7	14 ± 3·1
Sulphapyridine	13 ± 1·4	16 ± 3·0
Sulphisoxazole	11 ± 2·4	13 ± 2·5
Sulphadiazine	10 ± 1·4	10 ± 1·0
Propylene glycol controls	16 ± 2·4	18 ± 4·4

of oral and subcutaneous administration. The low K values for sulphadiazine and sulphisoxazole and the absence of toxic symptoms suggest that these two compounds are mild depressants. These results resemble closely those recorded for antibiotics[3] and suggest that the phagocytes do not play an important part in the action of these drugs. It is more likely that both antibiotics and sulphonamides act directly on invading organisms.

In the above investigations we gratefully acknowledge gifts of drugs from the Medical Directors of CIBA, Imperial Chemical Industries, Ltd., May and Baker, and financial assistance from the Central Research Fund of the University of London.

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Effect of Cobaltic Oxide Pellets on the Vitamin B_{12} Content of Ewes' Milk

Evidence that the provision of cobalt-containing supplements to ruminants will increase the vitamin B_{12} content of their milk is conflicting. Harper *et al.*[1] and Moinuddin *et al.*[2] found that cobaltized mineral mixtures given to ewes fed on dry rations significantly increased vitamin B_{12} levels in the milk. Other workers[3], however, have reported that supplementary feeding with cobalt-containing trace element mixtures had no effect on the vitamin B_{12} content of cows' milk when the animals were either stall-fed or grazed on pasture. According to Shrimpton and Duckworth[4] extra cobalt given to grazing ewes either as a drench or in a mineral supplement failed to increase the vitamin B_{12} content of the milk; but it seems doubtful whether any response would have been expected under the particular conditions of their trials.

In the work reported here a flock of pregnant ewes, grazing pastures marginally cobalt-deficient for lambs, was divided into two groups. Ewes in one group were each given a pellet containing 90 per cent cobaltic oxide (described by Dewey *et al.*[5]). Ewes in the second group served as controls. Lambing commenced 3 weeks later and continued for a further 3 weeks. When the lambs were approximately 3 months old and averaged about 50 lb. body-weight, and 5 weeks before weaning, milk samples were drawn from each group of ewes, extracted with cyanide[6], and assayed for vitamin B_{12} using *Lactobacillus leichmannii*[7]. Results are shown in Table 1.

Table 1

Group	No. of Ewes	Vitamin B_{12} (μgm./l.) Range	Mean
Cobaltic Oxide Pellets	15	4·3–19·1	10·3
Control	12	1·0– 4·6	2·5

The mean result for milk from pellet-treated sheep is comparable with mean values found by Harper *et al.*[1] for their cobalt-supplemented groups. Australian workers (O'Halloran, M. W., and Skerman, K. D., private communication) have also examined the effect of pellets on the vitamin B_{12} content of milk from pasture-fed ewes. Their results are similar to ours. It is concluded that continuous supplies of cobalt given in the form of cobaltic oxide pellets to grazing ewes will increase the vitamin B_{12} content of the milk several-fold.

According to Gregory[6], the vitamin B_{12} activity of milk is due almost entirely to cyanocobalamin, a form biologically active for higher animals. Hence it is of interest to consider to what extent ewe's milk will meet the lamb's requirement for the vitamin. On the basis of calculations made by Smith and Loosli[8], the daily requirement of a 50 lb. lamb for vitamin B_{12} given parenterally is about 9 μgm. But for crystalline vitamin B_{12} given orally existing evidence suggests that the daily requirement would not be less than 100 μgm.[8,9] and could be of the order of 300 μgm.[8] Lambs will drink about a litre of milk each day[10]. Thus, assuming that there is no great difference in availability to the animal between crystalline vitamin B_{12} and the bound form occurring in milk[6], it is evident that milk from the cobalt-supplemented ewe will provide only a small fraction of the lamb's total daily requirement for vitamin B_{12}.

The technical assistance of Messrs. A. J. Poole and B. J. Stephenson is acknowledged.

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Effects of Carbon Tetrachloride on Kidney and Liver Function in the Sheep

Carbon tetrachloride is often taken as a classical example of a hepatotoxin. However, there is some suggestion[1] that its lethal effect should be attributed to its action on the kidneys.

Five Corriedale adult wethers, between 35 and 42 kgm. body-weight, were drenched with a mixture of 50 ml. carbon tetrachloride and 100 ml. liquid paraffin. Estimations were made of bromsulphthalein clearance, p-aminohippurate synthesis from p-aminobenzoate and plasma concentrations of bilirubin and glutamate oxalacetate tranaminase as indications of liver function, and p-aminohippurate clearance and plasma concentrations of creatinine and urea as indications of kidney function. Methods used are described elsewhere[2].

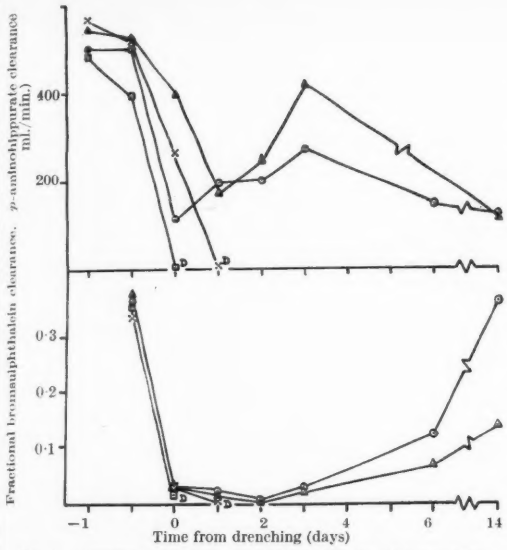

Fig. 1. Assessments of liver and kidney function in sheep after drenching with carbon tetrachloride. Each symbol (×, ◎, □ and △) refers to the same sheep throughout.

Two of the sheep remained clinically normal and three of the sheep died; one was found *in extremis* about 18 hr. after drenching, one died about 24 hr. and one 48 hr. after drenching. Symptoms noticed were progressive dullness and unwillingness to stand. Death occurred within 5 hr. of development of symptoms.

It will be seen from Fig. 1 that 18 hr. after drenching, there was, as expected, in all sheep a severe degree of liver dysfunction, as judged by fractional bromsulphthalein clearance. The other tests for liver function, plasma glutamate oxalacetate transaminase, plasma bilirubin concentration and *p*-aminohippurate synthesis from *p*-aminobenzoate also showed evidence of severe liver dysfunction. The maximum changes were found between two and three days after drenching (depending on the test) but function had returned virtually to normal by 14 days. In none of these tests was there any correlation between the severity of the dysfunction and the appearance of symptoms. There was also a decrease in *p*-aminohippurate clearance 18 hr. after drenching which persisted for the 14 days of the experiment. In those sheep which died, the *p*-aminohippurate clearance had fallen to very low values before death. In these sheep, there was also a rise in plasma urea and creatinine concentrations before death, but little change in the unaffected sheep.

These findings suggest that clinical symptoms and death were associated with almost complete cessation of kidney function in the presence of a severe degree of liver dysfunction. A similar degree of liver dysfunction with only a moderate degree of kidney dysfunction was not associated with clinical symptoms.

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Cartilage Homografts in Papain-injected Rabbits

Crude papain administered intravenously to young rabbits brings about ear collapse within 24 hours[1-4] and there is a concomitant loss of metachromasia of the ear cartilage[2]. A return to normal after a single dose occurs within a few days but can be delayed for about 28 days by the administration of cortisone[1]. Biochemical studies have shown that there is release of chondromucoprotein from the ear cartilage and a reduction of chondroitin sulphate content in the chondromucoprotein that remains in the cartilage[5]; there is also liberation, into the blood and urine, of a mucopolysaccharide resembling[6], in chemical and physical properties, chondroitin sulphates *A* and *C*. Cartilage homograft survival is held by some workers to be attributable to the mucopolysaccharide nature of the cartilage matrix[7,8]. Hence it was of interest to test survival of homografts of cartilage the matrix of which had been degraded by papain.

The papain sample used was found to produce ear collapse within 24 hours in each of three rabbits given 3 ml. of a 2 per cent aqueous solution intravenously. The ear cartilage from all three rabbits exhibited metachromasia which was, however, more alcohol-labile than that of normal ear cartilage. In the experiments to be described the papain effect was extended by administering cortisone to the recipients. As, however, cortisone has been shown to prolong survival of homografts[9,10,11,12,13], it was thought advisable, in order to counteract the latter property of cortisone, to use as hosts rabbits sensitized by skin homografts from the prospective cartilage donors. (It should be noted that these skin grafts were rejected by all the hosts in due course.)

Sixteen rabbits of under 1 kgm. body weight were used, eight as donors and eight as recipients. Each of four control recipients received a large homograft of ear skin, 14 days later a subcutaneous implant of a large piece of ear cartilage from the skin donor and then a daily intramuscular injection of 5 mgm. of cortisone for 28 days. The experimental group was dealt with in similar fashion with this difference that (*a*) the donor had papain-induced ear collapse when the cartilage was taken from its ear and (*b*) the recipient was given a dose of crude papain inducing ear collapse after implantation of the cartilage homograft. The cartilage homografts, sought after three months, were found to be rolled up but otherwise unchanged macroscopically in seven of the eight recipients. In the remaining rabbit, which was an experimental one, only a small hard nodule was recovered. The recovered material was fixed in Bouin's fluid, and embedded in paraffin. Sections were stained by (1) hæmatoxylin and eosin (2) toluidin blue and (3) the periodic acid-Schiff reaction.

Microscopically it was found that the fibrous nodule actually contained some small pieces of cartilage, and it is thought that in this instance infection had supervened. These cartilage remnants, and all other seven cartilage homografts recovered generally showed normal histological and histo-chemical staining properties.

These findings show that cartilage homografts the matrix of which had been degraded by papain at the time of transplantation and maintained in this condition for some time thereafter, persisted in sensitized hosts. The results are still reconcileable with the 'matrix' theory of cartilage homograft survival; for although much of the mucopolysac-

charide is removed by papain, the residual chondromucoprotein could well be sufficient to give protection to the graft.

I wish to express my thanks to Charles Zimmermann and Co. Ltd., Perivale, Middlesex, for their generous donation of the papain used in these experiments.

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Electrical Activity in the Muscle Cells of *Ascaris lumbricoides*

If a fresh specimen of *Ascaris* is opened by a longitudinal incision and pinned out under 30 per cent sea water at 37°C., fluctuating intracellular potentials can be recorded, using relatively large 3μ potassium chloride microelectrodes inserted into the swollen bodies of the large muscle cells.

Superimposed on the resting potential is a sequence of simple or complex depolarizing spikes normally of magnitude less than that of the resting potential.

If concurrent recordings are made of the potentials in two muscle cells, one of which is in the dorsal field and the other in the ventral, no correlation is found in the timing of the two sets of pulses (Fig. 1): if recordings are taken from two cells, a few mm. apart, in the same field, a definite correlation between the two records can be seen (Fig. 2). Each pulse in one record has its counterpart in the other. If the muscle cells concerned are at the same antero-posterior level, but at different distances from the nerve cord, the cell nearer the nerve cord gives the first pulse. If the cells are on a line parallel to the nerve cord, the anterior cell gives the first pulse.

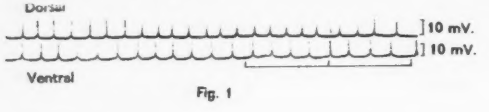

Fig. 1

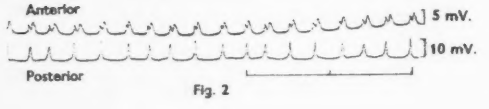

Fig. 2

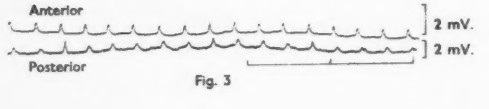

Fig. 3

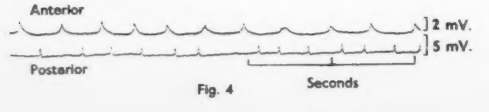

Fig. 4

Experiments involving transverse cuts made between an anterior and a posterior muscle cell have demonstrated two significant features.

(*a*) A transverse cut between the two cells, through the muscle column, hypodermis and cuticle of the worm, provided it does not sever the nerve cord, does not destroy the correlation between the pulses (Fig. 3).

(*b*) Cutting the nerve cord between the two cells, with or without an extension of the cut through the muscle column, abolishes the correlation, and independent sequences of impulses are then recorded from the two cells (Fig. 4).

Correlated pulses are therefore obtained so long as the nerve cord between the two cells is intact.

The origin of these depolarizing pulses and their relations to the membrane potential and tension in the muscle fibre are being investigated, but the results outlined above indicate the interesting possibilities of this material for neuro-physiological work.

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Distribution in the Mouse of Lethal and Sub-lethal Doses of Cottonmouth Moccasin Venom labelled with Iodine-131

Though disease and death from the bite of the poisonous snake is considered not unusual, and treatment has become more or less standardized, little is actually known about the site of action of snake venom *in vivo*. The value of a radioactive tag for studies of this sort is evident, and therefore, an attempt was made to label the whole dried venom of the cottonmouth moccasin (*Ancistrodon p. piscivorus*) with iodine-131, evaluate its toxicity relative to the original venom preparation, and chart its distribution in the mouse after injection of lethal or sublethal doses.

50 μgm. of whole dried venom, dissolved in physiological saline and buffered to a *p*H of 9, was labelled by alkaline extraction of cold carbon tetrachloride to which sodium iodide carrier and 3 mc. of iodine-131 had been added[1,2]. The extraction mixture was relieved of excess uncombined iodide and buffer by dialysis against dibasic potassium phosphate ($10^{-3}M$) at 34 C. for 48 hr. The resultant radioactivity of the labelled protein preparation was 117 c./m./μgm. (thin window Geiger-Müller counter).

Although the iodinated dialyzed venom was somewhat less toxic than whole venom, it was equally as toxic as whole venom which had been dialyzed but not iodinated (Fig. 1). This loss of toxicity of snake venom during dialysis has already been described by Goncalves[3], and is attributable to the loss of small molecules of toxic materials. A comparison between the toxic effects produced by the dialyzed venom and those caused by the non-hæmolytic toxins which are separable from the whole by ammonium sulphate precipitation indicates that enough of the hemorrhage-producing material is present in the dialyzed venom for it to contribute appreciably to the total effect.

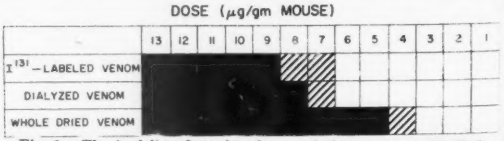

Fig. 1. The toxicity of *Ancistrodon p. piscivorus* venom labelled with iodine-131 compared with dialyzed venom and dried whole venom from the same sample. Black, complete lethal dose (100); hatched, minimum lethal dose; white, sub-lethal dose (0).

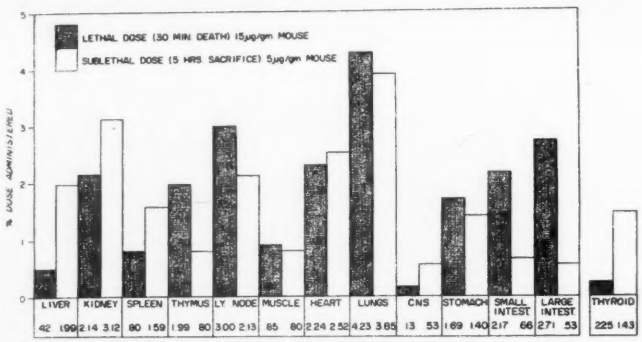

Fig. 2. The distribution of venom labelled with iodine-131 of the cottonmouth moccasin (*Ancistrodon p. piscivorus*) in the mouse.

Most mice injected intraperitoneally with 10–20 μgm. of iodinated-dialyzed toxin per gram of mouse died within 18 hr. and the remainder, which appeared in poor condition, died within 3 days following treatment. This is unlike the effect produced in the groups injected with the non-hæmolytic fractions in which the animals died within 24 hr. or not at all[4]. Further, the gross pathological changes produced by injection of the labelled venom were those which followed injection of the whole dried poison, namely, generalized hæmorrhage and œdema. The abdominal cavity and small gut were filled with serosanguineous fluid and the urine was bloody. All organs and tissues exhibited hæmorrhagic changes, most large vessels were thrombosed and the heart had stopped in systole.

The distribution of the labelled venom was observed after intravenous injection of mice at 2 dose-levels. The first, 15 μgm./gm., when injected into 9 20-gm. male Swiss mice caused death within 30 min. in all individuals; the second, 5 μgm./gm., when injected intravenously into 8 similar animals produced disease but no deaths, and within 5 hr. all showed significant improvement. Distribution of the radioactivity in pooled organ homogenates can be seen in Fig. 2. Before death the lethal dose did not accumulate to any great extent in the liver or spleen, organs in which collection or detoxification of venom might be expected to occur, even though some radioactive material presumably a metabolite, is present in the kidneys at this time. Relatively large quantities, however, were concentrated in the thymus and lymph nodes (mesenteric) but most striking was the large concentration of radioactivity in the lung. Though radioiodinated venom was not accumulated greatly by the skeletal musculature, a considerable quantity was found in the heart. Similar amounts were also found in the gastro-intestinal tract, especially in the colon. Only very small amounts of radioisotope were detected in tissues of the central nervous system.

The low thyroid radioiodide-level after 30 min. was interpreted as indicative of the integrity of the labelled venom protein.

It may be that the different distribution of radioactivity in tissues of animals which received a sublethal dose are due to the detoxification and/or excretion of the labelled protein in the period after administration. In support of this was the considerable increase in these animals of what was probably free radioiodide (thyroid) presumably accumulating as a result of the metabolism of the labelled venom protein. That the toxic properties of the latter have been reduced at this time was emphasized by the improved condition of the animal and its ultimate recovery. Concentration of the radioactivity in the lung had diminished and though it had increased in the heart it is difficult to say whether the slight change is significant. The increase in the radioactivity of the central nervous system, interpreted in the light of the complete absence of neurotoxic sign in the animal, is thought simply to be due to an accumulation of circulating radioiodide which has been shown to localize here[5]. If this were the case it might be indicative of a peripheral site for neurotoxic action. It is thought that the concentration in the lung and possibly the heart may be associated with the lethal effect of the venom. Further studies now under way seem to reinforce these preliminary data.

We are indebted to the Ross Allen Reptile Institute, Silver Springs, Florida, for the venoms used in this work and to H. P. Hall for assistance with the technical procedures.

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Passage of Insulin Through the Wall of the Gastro-intestinal Tract of the Infant Rat

It is well known that protein molecules can pass through the wall of the gastro-intestinal tract into the blood in new-born animals[1]. The possibility, therefore, exists that hormones which might be present in milk also are absorbed by infant animals. To test this hypothesis, insulin, which is ineffective when given *per os* to adult animals, was administered to infant rats by a stomach tube. Regular insulin was used in a dose of 40 units/ml.; 1 ml./100 gm. bodyweight. Rats were starved for 16–18 hours at 30° C. environmental temperature before the start of the experiment. Control animals received an equivalent dose of saline. All rats were killed two hours after insulin administration and blood glucose levels were determined by a modification of the Somogyi–Nelson method[2].

It is evident from Table 1 that in 2- and 8-day-old animals insulin causes a large drop in blood glucose levels when administered *per os*, whereas in 21- and 30-day-old animals this is no longer the case. This change in the hypoglycæmic effect of insulin occurs at the same postnatal period as the change in permeability to antibodies[1]. At this period alkaline phosphatase activity in the duodenum[1] and the whole intestine[3] increases and glucose absorption is also raised[3]. It is evident from Table 1, and has already been shown by others[4], that peptic activity in the stomach rises enormously between the 8th and 21st

Table 1. Hypoglycæmic Effect of Insulin Administered by Stomach Tube and Proteolytic Activity* of the Gastro-intestinal Tract

Age (days)	Weight (gm.)	Insulin (I.U.)	Blood sugar level (mgm. per cent)		Proteolytic activity	
			saline	insulin	stomach (units)†	intestine + pancreas (units +++)
2	4·5	2	39·3 ± 2·4 (8)	25·1 ± 2·1 (11)	0·01 (6 pooled)	0·81 (6 pooled)
8	10·3	4	75·0 ± 2·5 (10)	28·0 ± 2·5 (4)	0·00 (6)	0·23 ± 0·07 (8)
21	25·2	10	91·0 ± 4·5 (6)	95·0 ± 2·4 (6)	0·53 ± 0·15 (7)	2·10 ± 0·18 (7)
30	43·0	20	118·0 ± 9·8 (4)	136·0 ± 2·2 (5)	1·23 ± 0·16 (6)	4·00 ± 0·19 (6)

Values are given ± standard error of the mean. Figures in brackets are the numbers of animals.
* Proteolytic activity in the stomach was determined using biuret reaction at *p*H 2·1 (ref. 6). In the intestine and pancreas the amino-groups released at *p*H 9·2 were determined (ref. 7).
† 1 unit = 1 mgm. pepsin Organofarma (1 : 10,000).
‡ 1 unit = 1 mgm. pancreatin Organofarma (1 : 100).
Decrease of blood sugar level after insulin administration in 2 and 8-day-old animals are significant ($P<0.01$).

day. Tryptic activity of the intestine and pancreas also increases, but this increase is much less pronounced.

It was also tested whether insulin given subcutaneously to lactating rats would produce hypoglycæmia in the sucking infant animals. This could not be demonstrated. It was found, however, that lactating rats are very resistant to high doses of regular insulin (80–120 units per rat). Seven animals out of seven survived such a dose while 4 non-lactating female rats succumbed within 3 hr. of the injection.

It is also of interest that infant rats aged 2 and 8 days never had seizures when blood glucose levels were depressed, whereas it is reported that piglets do have seizures under such conditions[5].

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PLANT PHYSIOLOGY

Changes in Adenosine Di- and Tri-phosphate Concentrations in the Early Stages of the Action of of Yeast on Glucose

In a previous communication[1] we reported attempts to measure the concentrations of adenosine diphosphate and triphosphate in yeast fermenting or respiring in the presence of glucose, in the steady state of these processes. We concluded that there was little difference in the concentration of adenosine diphosphate under anaerobic or aerobic conditions, while the concentration of adenosine triphosphate was slightly higher aerobically. Propionitrile at a concentration sufficient to inhibit the Pasteur effect had no effect aerobically on the concentration of either substance.

A small technical improvement has now enabled us to identify with greater certainty the material in the ion-exchange chromatographic fractions which we had used to estimate adenosine di- and tri-phosphates, and we have applied this method to follow the changes in concentration of these substances in the early stages of fermentation and respiration in yeast.

25 ml. samples of a 30 per cent suspension (fresh weight/vol.) of bakers' yeast in *M*/40 *tris* buffer at *p*H 6·5 were bubbled at room temperature (approx. 20°) with purified nitrogen or with a vigorous stream of oxygen, and glucose solution added to give a final concentration of 1·2 per cent. After the appropriate time, with continuous gassing, 5 ml. of 50 per cent trichloracetic were added. After they had stood at room temperature for 1 hr., the suspensions were centrifuged and the yeast residue washed once with 20 ml. of 5 per cent trichloracetic acid. The combined extracts were treated with 2 gm. of barium acetate, the *p*H adjusted to 8·5, and the precipitate removed and dried.

The water-insoluble barium salt fractions were freed from barium by treatment with the ion-exchange resin 'Amberlite *CG*-120', neutralized with

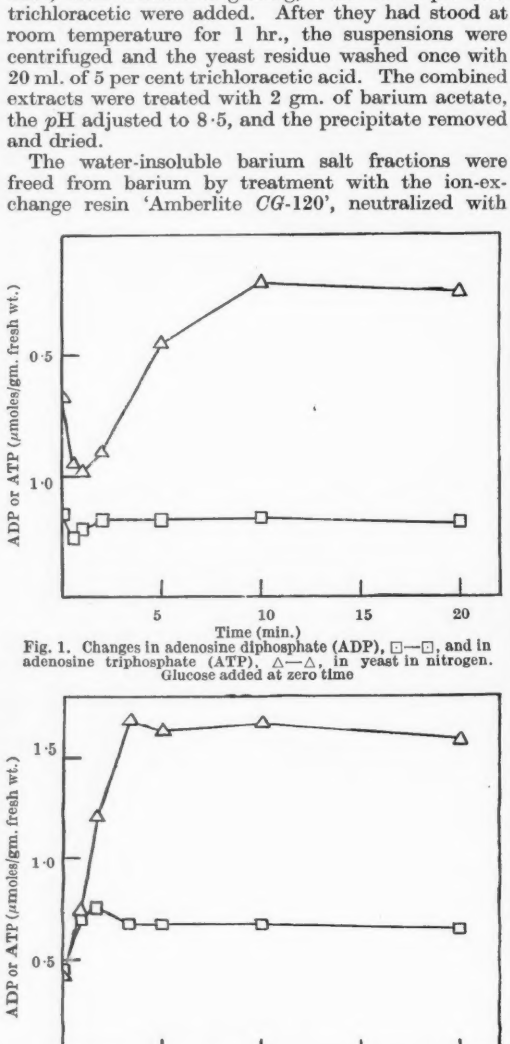

Fig. 1. Changes in adenosine diphosphate (ADP), ⊡—⊡, and in adenosine triphosphate (ATP), △—△, in yeast in nitrogen. Glucose added at zero time

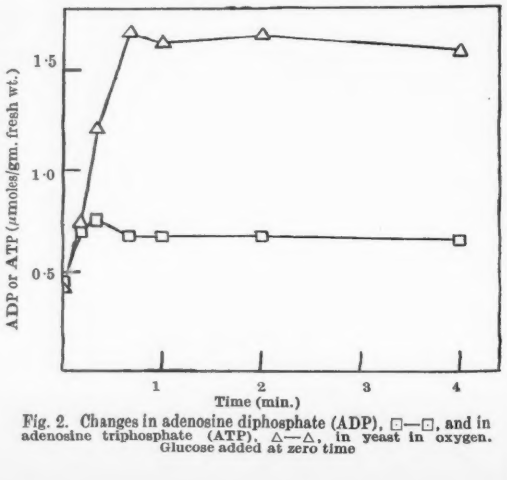

Fig. 2. Changes in adenosine diphosphate (ADP), ⊡—⊡, and in adenosine triphosphate (ATP), △—△, in yeast in oxygen. Glucose added at zero time

ammonia, and applied to a column of the anion-exchange resin 'Amberlite *CG*-400'[2]. The solutions used for elution from the resin column were hydrochloric acid 0·01 *N* + sodium chloride 0·002 *N* (*X*), hydrochloric acid 0·01 *N* + sodium chloride 0·02 *N* (*C*), and hydrochloric acid 0·01 *N* + sodium chloride 0·2 *N* (*D*). (*X*) removed the inorganic phosphate and much organic phosphate of a non-nucleotide nature which had previously interfered with the analysis of the adenosine di- and tri-phosphates, (*C*) removed adenosine diphosphate, and (*D*) adenosine triphosphate. The adenosine di- and tri-phosphate fractions obtained in this way gave molar ratios of adenine : ribose : phosphorus closely approximating to 1 : 1 : 2 and 1 : 1 : 3, respectively.

The result of a typical anaerobic experiment is shown in Fig. 1, and of a typical aerobic experiment in Fig. 2. Anaerobically, adenosine diphosphate fell to a minimum value after 1 min., then rose and reached its final steady value after 2 min. Adenosine triphosphate also fell initially, then rose more slowly and reached its steady high value in about 10 min. Aerobically, adenosine diphosphate rose to a small peak in 20 sec., and fell slightly to a steady value after 40 sec. Adenosine triphosphate rose steeply and reached its high steady value in 40 sec.

The final concentrations of both adenosine di- and tri-phosphates were practically identical, whether conditions were anaerobic or aerobic (average adenosine diphosphate, in nitrogen 0·31 μmole/gm. fresh weight, in oxygen 0·27 μmole; adenosine triphosphate, in nitrogen 1·26 μmoles, in oxygen 1·22 μmoles). This was found in three experiments with three different samples of yeast, though the steady-state concentrations of both nucleotides varied somewhat in the different experiments, as can be seen in the examples plotted in Figs. 1 and 2.

In all our experiments there was a large increase in the sum of adenosine di- plus tri-phosphate. The obvious source of this increase would be adenylic acid in the resting yeast, but our previous experiments[1] indicated that very little adenylic acid was present in the initial samples. We now find that adenylic acid, when in the presence of large amounts of other water-soluble alcohol-insoluble barium salts, does not behave normally on the ion-exchange column. Pure adenylic acid is completely eluted by 0·003 *N* hydrochloric acid (eluent *B* of Cohn and Carter[2]) but if adenylic acid is added to the alcohol-insoluble barium salts from a sample of yeast, and the mixture placed on the column, eluent *B* does not elute it. Instead, it appears in the eluate with eluent *X*, mixed with a number of other nucleotides which we have not been able to separate. However, the total quantity of this mixture, estimated in experiments with yeast such as those described above, shows a fall approximating to the rise in the sum of adenosine di and tri-phosphates, so there is little doubt that the sum of adenylic acid, adenosine di- and tri-phosphates remains, as would be expected, roughly constant.

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Response of *Sequoia sepervirens* (D. Don) Endl. and *Pseudotsuga menziesii* (Mirb.) Franco Seedlings to Temperature

Coniferous species show marked differences in their temperature requirements for seedling growth. These differences are connected with not only mean temperature but also with response to fluctuations in day, night and diurnal temperatures.

Kramer[1], working with *Pinus taeda* (L.) found that the plants made the maximum growth when the day temperature was 12 deg. C. or 13 deg. C. higher than the night temperature. Growth decreased as day and night temperatures approached equality regardless of the absolute level.

To determine if such diurnal temperature fluctuation is required by other conifers, we have grown redwood (*Sequoia sempervirens* (D. Don)) and Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), both from the northern coast of California, under a series of temperature conditions. One-month-old seedlings were grown for an additional 6 months in the Earhart Plant Research Laboratory under 16-hr. days of approximately 600-ft. candles intensity[2,3]. Twenty-four plants were grown per treatment.

We have found that the top growth of redwood seedlings responds significantly to day temperature (Fig. 1). Only when day temperature was low (7° C.) did increased night temperature significantly increase top growth. Root growth is, however, less responsive and is essentially not influenced over most of the range of temperature used. Cool nights (7° C.) with 23° C. day temperatures favoured root growth, whereas cool days (7° C.) with nights of 7° C. depressed root growth.

In contrast to the redwood, Douglas fir from the same region shows a marked optimum for top growth within the range of temperatures studied. This optimum was a 17° C. day temperature (Fig. 2) with both 7° C. and 23° C. night temperatures. Root growth exhibited the same optimum as well as better growth at a night temperature of 7° C.

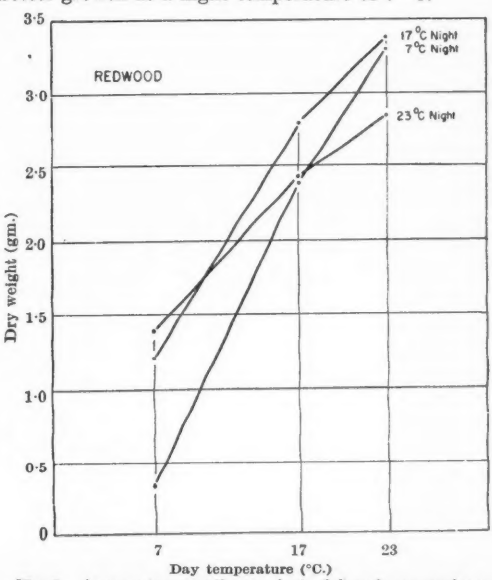

Fig. 1. Average top growth per plant of *Sequoia sempervirens* seedlings grown under nine different day-night temperature conditions.

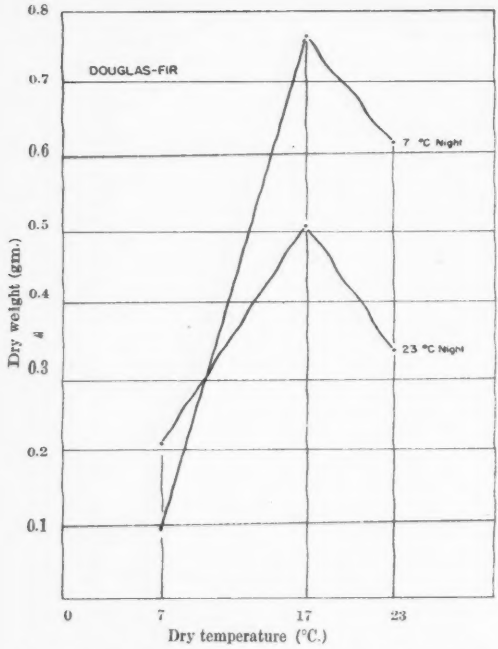

Fig. 2. Average top growth per plant of *Pseudotsuga menziesii* seedlings grown under six different day-night temperature conditions.

Thus, these results show that a diurnal temperature variation is not required for the maximum growth of redwood. The case is not so clear for Douglas fir; this plant makes optimum growth with a 10 deg. C. diurnal variation; but a diurnal variation of 16 deg. C. inhibits growth.

The fact that redwood initially grows much faster than Douglas fir (Figs. 1, 2) has been noted by others working with these species[4].

Further studies are being conducted with extended temperature ranges to obtain additional information on the relative effects of temperature of the growth of these and other conifers. More detailed reports will be presented elsewhere as the individual studies are completed.

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PLANT PATHOLOGY

Some New Specific Bacteriophages for Plant Pathogenic *Xanthomonas* spp.

The study of phages from the point of view of plant pathology has practical applications besides being of theoretical interest. By the use of specific phages the detection of plant pathogenic bacteria was possible in infected seeds and tissues. Furthermore, using phages, slight biological differences were demonstrated between pathogenes otherwise hardly distinguishable from each other. Therefore, the detection and isolation of new specific phages has considerable interest. The present communication will deal with the isolation of some new phages for *Xanthomonas* spp.

Xanthomonas carotæ (Kendrick) Dowson was first described as a pathogen damaging the leaves and influorescences of carrot in Europe[3]. Heavily infected leaves and inflorescences were ground and from the ground material a bacteriophage, specific for *X. carotæ*, was isolated. On agar plates the phage for *X. carotæ* forms plaques of 1–2 mm. in diameter. Of the 30 *Xanthomonas* species tested only strains of *X. carotæ* were lysed by the phage, which indicates a high specificity (Table 1).

Table 1. Specificity of Phages for *X. carotae* and *X. vesicatoria* from Tomato and Pepper

Organism tested	Phage for *X. vesicatoria* from tomato	Phage for *X. vesicatoria* from pepper	Phage for *X. carotæ*
X. Vesicatoria			
(1588) from pepper ..	−	+	−
(1596) ,, ..	−	+	−
(1608) ,, ..	−	+	−
(801) from tomato ..	+	−	−
(802) ,, *	−	−	−
(803) ,, *	−	−	−
(804) ,, ..	+	−	−
(806) ,, ..	+	−	−
(807) ,, ..	+	−	−
(808) ,, ..	+	−	−
(809) ,, ..	+	−	−
(810) ,, ..	+	−	−
(424) ,, †	+	−	−
X. carotæ (No. 15)	−	−	+
(No. 66)	−	−	+
(No. 78)	−	−	+
X. begoniæ (*XB* 10)‡ ..	−	−	−
X. campestris (*XC* 107)‡ ..	−	−	−
X. citri (407)†	−	−	−
Ps. gardneri§	−	−	−
X. hyacinthi (*XH* 104)‡ ..	−	−	−
X. malvacearum	−	−	−
X. pelargoni (*XP* 136)‡ ..	−	−	−
X. phaseoli (*E*1388) ..	−	−	−
var. fuscans (*E*1299) ..	−	−	−
X. ricinicola (113)† ..	−	−	−
X. stewarti (449)†	−	−	−
X. translucens (*XT* 19)‡ ..	−	−	−
X. uredovorus (*O* 049) ..	−	−	−
X. rasculorum (181)† ..	−	−	−

* The strains *S*2 and *S*03 also differ in their other biological properties from the other strains isolated from tomato.
Strains supplied by :
† National Collection of Plant Pathogenic Bacteria, England.
‡ M. P. Starr, Univ. of California, Davis.
§ D. Sutic, Institut for Plant Protection, Beograd, Yugoslavia.

Xanthomonas vesicatoria (Doidge) Dowson is a pathogen of tomato and pepper (*Capsicum annum*) widespread in Hungary[2]. Hitherto the bacteria isolated from the diseased tomato and pepper plants have been regarded as a uniform species. Only a recent study by Sutic[4] revealed the differences between the pathogens concerned. By the use of specific phages we also were successful in proving the differences between the two bacteria isolated from tomato and pepper.

The phage isolated from infected tomato fruits lysed exclusively the bacteria deriving from tomato, without affecting the pathogens of pepper. The clear plaques formed by this phage are 1·5–2 mm. in diameter.

Similarly, strongly infected pepper leaves contained another phage which lysed only the pathogens deriving from pepper. The bacteria isolated from tomatoes were resistant to it. This phage formed plaques of varying diameter (1–3 mm.).

The two phages were assayed for eventual infectivity against 19 *Xanthomonas* species. All of these proved to be resistant (Table 1).

The conclusion can be drawn that all the three phages exhibit a high degree of specificity. The last two make it possible to detect variants within the *X. vesicatoria* species.

It has thus been demonstrated that the species *X. vesicatoria* is not really uniform, that is, the pathogen isolated from the tomato plant is not identical with that occurring on pepper. The work carried out with the two specific phages justifies the conclusion of Burkholder and Li[1] and Sutic[4], based on slight biochemical differences, as to the different nature of pathogens damaging tomato and pepper.

Further studies on the biological properties and mutual relations of various pathogens belonging to *X. vesicatoria* group will be published elsewhere.

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ANIMAL PATHOLOGY

Urinary β-Glucuronidase Activity in Patients with Bone Fractures

DURING an investigation into the urinary β-glucuronidase activity of patients with cancer of the bladder a substantial number of controls was investigated. These controls were grouped into normal adults and patients with pathological conditions other than cancer of the bladder. Among them was a group in hospital with fractures of bone.

24-hr. specimens of urine were collected and the method of estimating the β-glucuronidase activity was a slight modification of that used by Boyland, Gasson and Williams[1]. The results were expressed as units of activity per ml. of urine and from this was calculated the enzyme excretion per day. Results from the following groups of patients are summarised in Table 1.

Group 1. Normal control subjects.

Group 2. A 'miscellaneous' group of patients excluding those with genito-urinary diseases.

Group 3. Patients who within the past eight days had undergone minor surgery.

Group 4. Patients who within the previous eight days had undergone major surgery in which some trauma and subsequent repair might be expected.

Group 5. Patients who within the previous fourteen days had suffered bone fractures.

In these five groups only those results were included in which the *p*H of the urine was within normal limits and the patient was not pyrexial during the urine collection.

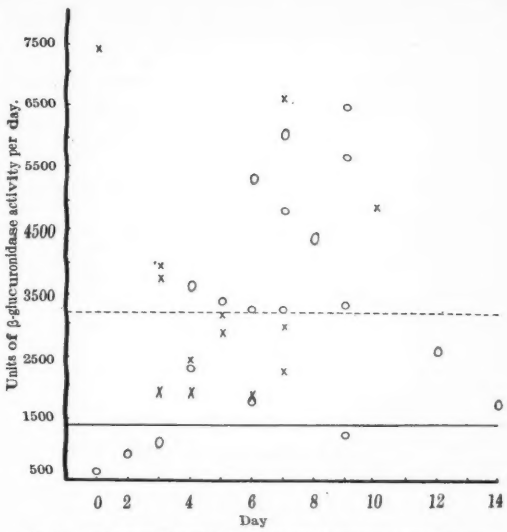

Fig. 1. Daily excretion of β-glucuronidase in patients after fractures or the leg or after major operations.
The average value for normal subjects is ———— with twice the standard deviation — — — — —.
O, patients with fractures; ×, patients after a major operation.

The results indicated that the urinary β-glucuronidase activity was significantly raised after major operations. This is in accord with the observation[2] that the urinary enzyme activity is increased immediately after operations. On the other hand, minor operations did not result in any such elevation. In the group of patients with fractures, the urinary β-glucuronidase activity was also significantly increased.

This fracture group included eighteen patients with fractured lower limbs and only four with fractured arms. It is interesting, though not statistically significant, that in these four, although serious enough to be kept in hospital, the enzyme activity was not increased to the level of those with fractured legs. Fig. 1 demonstrates that in lower-limb fractures, the urinary β-glucuronidase reached a peak 6–9 days after the accident and then fell.

The apparent relationship between the height of the β-glucuronidase activity and the degree of trauma and subsequent repair may be more than coincidental and is being investigated more fully. It is possible that this activity may be associated not only with hydrolysis of chondroitin[3] but also with active formation of new bone.

Table 1. URINARY β-GLUCURONIDASE ACTIVITY

Group	*n*	β-glucuronidase activity—units/ml. of urine			't' test against normal subjects		β-glucuronidase activity—units/day			't' test against normal subjects	
		mean	*S.D.*	*S.E.* of mean		*P.*	mean	*S.D.*	*S.E.* of mean	*t*	*P.*
1 Normal Subjects	32	1·05	0·55	0·098	—	—	1405	893	160	—	—
2 'Miscellaneous' diseases ..	55	1·16	0·62	0·084	0·82	between 0·5 and 0·4	1719	1047	141	1·40	between 0·2 and 0·3
3 After minor surgery	20	1·19	0·39	0·088	0·98	between 0·4 and 0·3	1523	569	127	0·52	0·6
4 After major surgery	13	3·72	1·68	0·466	8·08	less than 0·01	3534	1750	486	5·37	less than 0·01
5 After bone fractures	27	2·55	1·24	0·239	6·14	less than 0·01	2990	1740	323	4·48	less than 0·01

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Production of an Agglutinating Auto-Antibody (Panagglutinin) Active upon Tanned Erythrocytes in the Rabbit

P. Levine *et al.*[1] demonstrated the positive immune response of the rabbit to the injection of tanned *hetero-* or *iso*-erythrocytes coated with *P*—reactive material obtained from hydatiform cysts. This response is rapid and vigorous, contrasting with the slow or negative result when the same material was injected by other routes, even with the use of adjuvants.

We used this technique for the introduction of several soluble antigens into the rabbit, especially human gamma-globulin plasma fraction and thyroglobulin obtained from fresh human thyroids[2]. The results collected to date show that the intravenous injection in a rabbit, of iso-specific tanned erythrocytes coated with the desired antigen often leads quickly to a very spectacular immune responses. For example, the production of specific anti-human globulin by this method was found to be the most satisfactory of all the methods in our experience.

Blood was obtained from the ear vein of a group of three 'donors' (Nos. 15–17). The erythrocytes were, washed three times in phosphate in phosphate-buffered saline at *p*H 7·2, resuspended to a concentration of 4 per cent, mixed with one volume of a 1/20,000 tannic acid solution in buffered saline, shaken gently at room temperature for 10 minutes, washed once and re-suspended to a concentration of 2 per cent in physiological buffer to which 1 per cent of normal rabbit serum had been added. One volume of this suspension was mixed with one volume of a 0·4 per cent solution of human gamma globulin and allowed to stand for 30 minutes. The erythrocytes coated with human γ-globulin were then washed free from any excess of γ-globulin and readjusted to a concentration of 2 per cent in physiological buffer containing 1 per cent normal rabbit serum.

Table 1. Neutralization Study of the Rabbit Serum *T*γ III*

Test cells:	Original serum					Serum neutralized with human gamma-globulin				
	2	4	8	16	32	2	4	8	16	32
Rh-positive human red cells: Normal	—	—	—	—	—	—	—	—	—	—
Rh-positive human red cells: Sensitized	(3)†	(4)	(5)	(5)	(5)	—	—	—	—	—
Tanned auto-erythrocytes coated with human γ-globulin	(2)	(2)	(4)	(4)	(4)	(2)	(3)	(2)	(1)	—
Tanned auto-erythrocytes	(3)	(3)	(2)	(1)	—	(3)	(2)	(2)	+	—

* One part of the serum was neutralized with one part of 0·4 per cent human gamma-globulin.
† (3) = +++, (4) = ++++, etc.

A group of three rabbits were injected intravenously, twice weekly, with 2 ml. of this suspension. After four injections (on the fourteenth day of immunization) the titre of the agglutinin was well above 1/2,000 against rabbit erythrocytes coated with γ-globulin and against human Rh-positive sensitized cells.

After twelve intravenous injections of the same material (that is, at the end of the sixth week), the sera of all three rabbits exhibited prozones and reached an anti-globulin agglutination titre above 1/16,000. In addition, the three rabbits had developed specific iso-agglutinins against blood groups present in some of the 'donors' and absent on their own erythrocytes.

Finally, one of the rabbits (*T*γ III) had developed an agglutinin active on all tanned red cells of the

Table 2. Absorption Study of the Rabbit Serum *T*γ III *d.**

Test cells:	Unabsorbed					Absorbed with γ-globulin-coated erythrocytes					Absorbed with pooled normal iso-erythrocytes (No. 16 and No. 17)					Absorbed with tanned auto-erythrocytes (*T*γIII)					Antibody tested
	2	4	8	16	32	2	4	8	16	32	2	4	8	16	32	2	4	8	16	32	
Rh-positive human red cells: Normal	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Anti-human -β-globulin
Rh-positive human red cells: Sensitized†	(3)‡	(4)	(5)	(5)	(5)	—	—	—	—	—	(3)	(4)	(4)	(4)	(4)	(3)	(4)	(4)	(4)	(4)	Anti-human -β-globulin
Normal iso-erythrocytes (No. 16)	(2)	+	±	—	—	(2)	+	±	—	—	±	—	—	—	—	(3)	(2)	+	—	—	Iso-antibody
(No. 17)	(4)	(2)	(2)	±	—	(4)	(2)	(2)	+	?	±	—	—	—	—	(3)	(2)	(2)	±	—	Iso-antibody
(No. 15)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Iso-antibody
Normal auto-erythrocytes (*T*γIII)	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	Iso-antibody
Tanned iso-erythrocytes (No. 16)	(4)	(3)	+	+	—	(4)	(3)	+±	+	—	(4)	(4)	(2)	+	—	±	—	—	—	—	'Auto-antibody' against tanned erythrocytes
(No. 17)	(5)	(4)	(4)	(2)	+	(4)	(4)	(4)	(2)	±	(4)	(4)	(3)	+	±	(2)	+	—	—	—	'Auto-antibody' against tanned erythrocytes
(No. 15)	(3)	(3)	(3)	+	—	(4)	(4)	(3)	+	—	(4)	(4)	(3)	+	—	±	—	—	—	—	'Auto-antibody' against tanned erythrocytes
Tanned auto-eythrocytes (*T*γIII)	(3)	(3)	+±	±	—	(3)	(3)	+±	+	—	(4)	(3)	+	±	—	±	—	—	—	—	'Auto-antibody' against tanned erythrocytes
Tanned auto-erythrocytes coated with human γ-globulin	(4)	(4)	(4)	(4)	(4)	(3)	(2)	+	+	—	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)	Anti-human γ-globulin

* Absorption was performed with 1 vol. of packed cells for 3 vol. of serum, at 37° C. for 20 minutes.
† Rh-positive human red cells of genotype *CDe/CDe* were sensitized by 5 volumes of very potent anti-*C* + *D* and washed six times in buffered saline.
‡ (3) = +++, (4) = ++++, etc.

rabbit, including auto-erythrocytes, up to a titre of 1/16—1/32. Neutralisation with human gamma-globulin showed that the auto-antibody was independent (Table 1).

Suitable absorption experiments (Table 2) with erythrocytes coated with human-gamma-globulin, with heterospecific *iso*-erythrocytes, and with tanned auto-erythrocytes eventually showed that three distinct and independent antibodies were present in this particular serum ($T\gamma$ III): (1) a high titre anti-human-γ-globulin; (2) an iso-agglutinin active on a rabbit blood group antigen; (3) an auto-agglutinin (pan-agglutinin) active on tanned rabbit erythrocytes.

A curious phenomenon may be observed when the serum has been absorbed with tanned auto-erythrocytes (Table 2): tannic acid appears to destroy or inhibit the agglutination of iso-erythrocytes No. 16 and No. 17 by the iso-antibody, although it apparently had no effect on their antigenicity, as shown by the iso-immune response in rabbit $T\gamma$ III into which they were injected.

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BIOLOGY

Endogenous Rhythms of Body Temperature in Hibernating Bats

Endogenous rhythms of activity with periods close to 24 hr. have been described previously in the two most common bats of the north-eastern United States: *Myotis lucifugus* and *Eptesicus fuscus*[1,2,3]. Rawson has shown that this periodic activity persists for at least several weeks under conditions of constant darkness and temperature. Folk[4] has suggested that the endogenous "clock" may control the timing of the periodic arousals of bats during their winter hibernation.

Recent experiments in this laboratory have demonstrated that the endogenous clock of both *Myotis lucifugus* and *Eptesicus fuscus* continues to function, with a period in the neighbourhood of 24 hr., while the animals are in hibernation in constant darkness and at various constant ambient temperatures of 3°–10°C.

Bats were taken from an abandoned mine in Hibernia, New Jersey, in which they were hibernating and kept, with drinking water but no food, in an ordinary refrigerator at about 6°C. Under these conditions, they returned quickly to hibernation. For the experiments, the bats were removed from the refrigerator, their feet were held in a felt-lined clamp, a rectal thermocouple was inserted, and they were placed in a moist chamber. The moist chamber was then placed in a precision temperature-controlled cabinet in constant darkness. A sensitive Brown recording potentiometer (full scale = 60 μV.) was used to record the difference between the bat's rectal temperature and that of a reference junction in the air of the moist chamber. The temperature of the moist chamber was also monitored independently on another channel.

Figs. 1 and 2 show the kinds of rhythmic fluctuations in body temperature obtained from bats in constant darkness and at constant ambient temperatures of 8° to 10°C. The bat, the record of which is

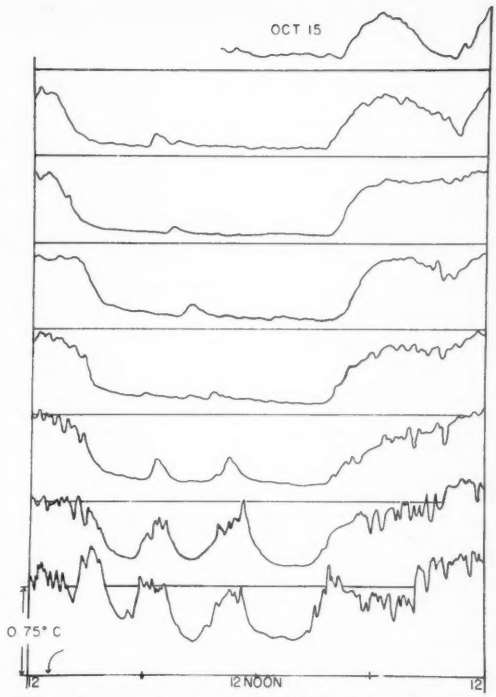

Fig. 1. Continuous record of the rectal temperature of a female *Myotis lucifugus* in constant darkness and at an ambient temperature of 10°C. The height of the temperature record above the base line is a measure of the difference in temperature between the bat and its environment. Successive days are plotted under each other.

shown in Fig. 1 was collected on October 7 and the experiment began on October 15. As is shown in Fig. 1, its body temperature never rises more than 1° above the ambient and there are several distinct features of the curve which recur with somewhat different periods. The bats, the records of which are shown in Fig. 2 were collected on January 28 and placed in the experimental apparatus on February 3 and 6. The extreme sensitivity of the recording potentiometer did not permit following the body temperature further than 1½ deg. above ambient temperature. However, measurements made with a potentiometer of lower sensitivity on other bats indicate that, when the body temperature rises quickly and smoothly as in Fig. 2*A* and on 5 of the days shown in Fig. 2*B*, a body temperature rise of at least 15°C. and more often 20°–25°C. is indicated. The differences in form and amplitude between the temperature record shown in Fig. 1 and those shown in Fig. 2*A* and *B* seem to be dependent on the length of time the animals have been in hibernation and not on the sex or species. Autumn animals (those collected soon after entering hibernation in the autumn) always show temperature records similar to Fig. 1, whereas winter bats, which have been in hibernation a month or more, show records similar to those in Fig. 2. It is possible to obtain records similar to those of autumn animals by arousing a bat in February, keeping it in a warm room, feeding it for several weeks, and then measuring its body temperature after returning it to hibernation.

A consistent feature of the temperature records of winter bats (such as those in Fig. 2) is that on some days the temperature elevation is large, on other days

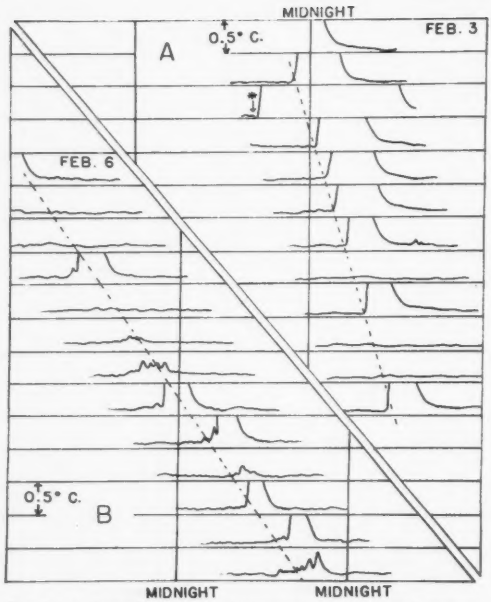

Fig. 2. *A*, Record of the rectal temperature of a female *Eptesicus fuscus* in constant darkness. The ambient temperature was 7·5°C. until the asterisk when it was raised to 9°C. where it remained for the duration of the experiment. *B*, Record of the rectal temperature of a female *Eptesicus fuscus* in constant darkness at constant ambient temperature of 9°C.

it is very small and occasionally it does not occur at all. However, the rhythmic process which controls the timing of the temperature elevation continues to function independently of the elevation itself. This is illustrated by the way in which all the elevations fit a straight line regardless of whether or not they have been preceded by a day or two in which the animal's body temperature remained constant.

The fact that the bat clock continues to function with a period close to twenty-four hours at a body temperature of 8°–10°C. demonstrates its temperature compensated nature. Most of the records obtained from hibernating bats show periods longer than 24 hr., whereas Rawson's observations on activity of bats at room temperatures indicate that, under these conditions, the period of the bat clock is shorter than 24 hr. It is therefore probable that temperature does affect the period of the bat's clock, but to a very slight degree. Although bats are not completely homœothermic, the fact that their clocks have the fundamental feature of temperature compensation in common with the clocks of poikilotherms may make useful a comparison of the endogenous timing systems in these two widely different groups.

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ENTOMOLOGY

Copulation of *Glyptotendipes (Phytotendipes) paripes* Edwards

An investigation of the ethology of this chironomid has been carried out by a team of investigators from the Entomological Research Center of Florida State Board of Health, and a report of the results is under preparation. During this work it was found that the males frequently carried a spermatophore between the claspers. This position is an artefact as the spermatophore is normally carried inside the genital tract of the male, but when killed it is ejaculated into a position which probably is the one used during the copulation.

The spermatophore consists of a rather hard gelatinous mass about the shape of the seed of a birch tree ; it is flat with a rounded base and a pointed apex (Fig. 1). In the basal part are two cavities in which the thread-like spermatozoa can be seen. From each cavity runs a twisted tube to the apex.

Fig. 1. Spermatophore of a male *Glyptotendipes* (*Phytotendipes*) *paripes*.

The copulation may be initiated and probably also completed in flight ; or it may begin in the air and be finished on some object ; or it may be completely consummated while the animals are on a solid base.

The male crawls over the female from behind, the tips of the abdomens make contact and the male drops back for a moment hanging head down. The process is difficult to observe in detail as it only lasts about 3–5 sec.

The male hypopygium is not inversed as in Culicidae ; but the last segments of the abdomen are extremely flexible as the 8th segment is somewhat petiolated.

The spermatophore is placed in the female in the genital opening between the 8th and 9th sternite. There is here a cavity with a foramen in the center of a chitinized, pelvis-like structure. From this foramen run cranially the ducts to the two spermathecæ which are located under the two light coloured areas of the 8th sternite. The spermatophore is placed with the apex touching the foramen and the base protruding behind the posterior edge of the 8th segment.

The spermatophore is first pink, and in such cases the spermathecæ are still empty ; it is the sperm which have the reddish colour ; in individuals in which the sperm have moved to the spermathecæ these organs have a colour of deeper pink than do those of virgins, while the spermatophore becomes white.

How long a time it takes from the placing of the spermatophore until the sperm are transferred to the spermathecae is unknown; but is probably of the order of 5–20 min. As the spermatophore completely blocks the genital orifice, only one copulation is possible. The virgin has a tendency to curve the tip of the abdomen up while the inseminated female curves the tip downwards, especially when it is refusing an attempt at copulation by a male. The males will attempt to copulate again a few seconds after a completed mating; but whether such attempts are followed by actual insemination is unknown.

The copulation of this or closely related species seems not to have been described before. The process itself did not seem to differ much from copulation in other *Nematocera* except for the unexpected finding of a transfer of the sperm by means of a highly developed spermatophore. No reference to this way of copulation has been found in the literature consulted[1,2,3]. Imms[4] states that "Spermatophore formation . . . apparently never occurs in the Diptera (Khalifa, 1949)[5]".

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Esterase Inhibition and Organophosphorus Poisoning in the House-Fly

IN a recent review paper Winteringham and Lewis[1] stated that although inhibition of cholinesterase constitutes a major biochemical lesion in insects exposed to organophosphorus compounds, the importance of other lesions should not be under-estimated. Evidence of such a lesion has been found in the inhibition of other esterases. Van Asperen[2] has shown that in the early stage of poisoning of house-flies treated with O,O-dimethyl-O-2, 2-dichlorovinylphosphate (DDVP) the aliphatic esterase hydrolysing methylbutyrate was inhibited by 83 per cent, whereas the inhibition of cholinesterase was only 27 per cent. He suggested that the high inhibition of the aliphatic esterase was more important than cholinesterase inhibition in organophosphorus poisoning. Hopf and Taylor[3] arrived at a similar conclusion when investigating organophosphorus poisoning in the locust.

These investigators[2,3] used lethal doses of organophosphorus compounds. During a study of sub-lethal effects of tetraethylpyrophosphate (TEPP), an attempt was made to classify the esterases of the house-fly, *Musca domestica* L. The method used was that of Mounter and Whittaker[4] for selective inhibition of esterases. Determinations of esterase activity in homogenates of three-day-old female house-flies were carried out manometrically at *p*H 7·4 and at 25° C. according to the method of Augustinsson[5]. The substrates used were acetylcholine, triacetin, phenylacetin, phenylacetate and ethylbutyrate. Eserine at 10^{-5} *M* was used for the *in vitro* inhibition of cholinesterase, while TEPP at 10^{-6} *M* was used for inhibition of aliphatic esterase and cholinesterase.

It was shown that acetylcholine was hydrolysed only by cholinesterase, and ethylbutyrate only by ali-esterase. Triacetin and phenylacetate were hydrolysed by both cholinesterase and ali-esterase, and also by a TEPP-insensitive esterase, presumably an aromatic esterase[6].

Experiments carried out with mixed substrates showed that ethylbutyrate, triacetin and phenylacetate were hydrolysed by the same aliphatic esterase. This enzyme hydrolysed methylbutyrate at a rate only slightly less than ethylbutyrate, and is presumably identical with the aliphatic esterase reported by Van Asperen[2]. The ali-esterase had a well-defined optimum substrate concentration at $1{\cdot}7 \times 10^{-2}$ *M* ethylbutyrate.

Because Van Asperen[2] worked with homogenates of whole flies rather than with isolated nervous tissue, he was unable to make a statement about the localization of the different esterases. An examination of the esterase content of the isolated thoracic ganglion of the house-fly revealed that 91 per cent of the total cholinesterase of the thorax was found to be present in the ganglion. Of the total ali-esterase activity of the thorax, only 4 per cent was found in the ganglion. This finding may be of some importance in determining the site of organophosphorus poisoning in the fly.

Both cholinesterase and ali-esterase were equally sensitive to inhibition by TEPP *in vitro*, the pI_{50} being 8·9, when the enzymes were incubated with the inhibitor for 1 hour at 25° C. Under similar conditions the ali-esterase was much more sensitive to tri-*o*-cresylphosphate (TOCP) than was cholinesterase. TOCP at $2{\cdot}5 \times 10^{-5}$ *M* caused 50 per cent inhibition of ali-esterase, whereas cholinesterase remained uninhibited. By injection or topical application of a chosen dose of TOCP, it proved possible to inhibit *in vivo* almost all the ali-esterase activity without appreciably affecting that of cholinesterase. Flies treated in this way did not show any symptoms of poisoning.

These results indicated that it should be possible to inhibit the aliphatic esterase of the fly, and then to determine the effect of subsequent treatment with TEPP upon knock-down and the inhibition of cholinesterase. Flies were treated topically with 90 μgm. of TOCP. Twenty-four hours later, a sample of these flies were treated topically with 0·2 μgm. of TEPP. Control flies received the same dose of TEPP. Both samples of flies were prostrated by the TEPP treatment. The effect of these treatments upon the inhibition of the cholinesterase and ali-esterase is given in Table 1. Prior to treatment with TEPP, the ali-

Table 1. EFFECT OF TRI-*o*-CRESYLPHOSPHATE (TOCP) AND TETRAETHYLPYROPHOSPHATE (TEPP) UPON CHOLINESTERASE AND ALIPHATIC ESTERASE ACTIVITY IN THE HOUSE-FLY

Treatment	Cholinesterase activity b_{22}/fly	Cholinesterase inhibition (per cent)	Aliphatic esterase activity b_{22}/fly	Aliphatic esterase inhibition (per cent)
None (controls)	57	—	162	—
90 μgm. TOCP	59	0	31	81
90 μgm. TOCP + 0·2 μgm. TEPP	19	67	10	94
0·2 μgm. TEPP	28	51	6	96

Female house-flies were treated with TOCP; after 24-hr. esterase activities were determined in samples of treated and control flies, using acetylcholine and methyl-*n*-butyrate as substrates. Two other samples of TOCP-treated and control flies were treated with TEPP and the esterase activities were determined at the time prostration occurred.

Esterase activities are expressed as b_{22}, that is, μl. of carbon dioxide evolved per 30 min. Each figure represents the mean of 4 determinations.

phatic esterase of the flies treated with TOCP was inhibited to a large degree. The cholinesterase was uninhibited. Treatment with TEPP of the sampel of TOCP-treated flies resulted in inhibition of the cholinesterase at prostration. Treatment of the control flies with TEPP alone caused high inhibition of the ali-esterase and inhibition of the cholinesterase. This latter result is somewhat similar to that obtained by Van Asperen[2] for flies treated with DDVP, but since TOCP-treated flies can tolerate, without showing apparent symptoms of poisoning, a high level of aliphatic esterase inhibition, it is doubtful that this enzyme alone is important in organophosphorus poisoning. The evidence in Table 1 appears to refute Van Asperen's[2] suggestion in this respect. The present work strongly indicates that the inhibition of the aliphatic esterase is of minor importance when compared to the inhibition of cholinesterase. At the same time, it shows that the level of cholinesterase inhibition causing knock-down of the flies is important when evaluating cholinesterase on this basis.

This work will be published more in detail elsewhere.

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BACTERIOLOGY

Enhancement of Pigment Production by Pseudomonas

One of the principle factors to be considered in the preparation of media for the detection of fluorescent pigments by *Pseudomonas* is the content of available iron. When Totter and Moseley[1] tested oxine (8-hydroxyquinoline) in an effort to lower the metal content of media by chelation they failed to demonstrate an increase in pigment formation. Despite this rather unexpected result the following method was evolved which, on testing with a wide range of pseudomonads, proved highly successful in stimulating pigment production from many organisms which were previously considered negative or very weakly positive in this character.

The constituents for 1 litre of the medium were :

Dihydrogen ammonium phosphate	1·0 gm.
Potassium chloride	0·2 gm.
Crystalline magnesium sulphate	0·2 gm.
Potassium or sodium gluconate	5·0 gm.

These were dissolved in 400 ml. of glass-distilled water with 2 ml. of 0·5 per cent w/v 8-hydroxyquinoline in redistilled chloroform[2] and agitated vigorously in a separating funnel. The chloroform layer was allowed to settle out and then removed. This process was repeated three further times with fresh chelating agent. Finally the solution was washed twice with 5 ml. quantities of redistilled chloroform to remove the excess oxine. After the removal of the visible traces of chloroform the solution was made up to 1 litre with glass-distilled water, adjusted to *p*H 7, dispensed in normally cleaned tubes and sterilized at 22 lb. pressure.

If a solid medium was desired, shred agar was subjected to a similar chelating process. The unmelted agar was treated at the same time with two solutions; the oxine-chloroform (as above) and a 0·1 per cent solution of ethylenediaminetetraacetic acid (sodium salt) in water. The reagents were replaced every day for 5 or 6 days and the agar finally washed thoroughly with chloroform and water. When dried the treated agar was used in normal concentration to solidify the gluconate medium.

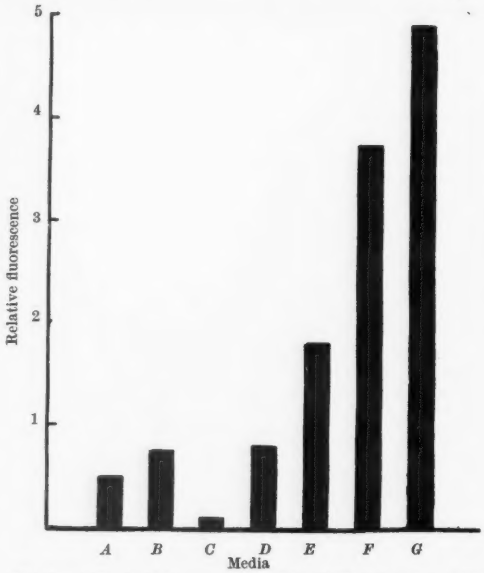

Fig. 1. A fluorimetric comparison of the ability of various solid media to support fluorescent pigment production by a *Pseudomonas* (arbitrary scale).
A, peptone Lemco agar (uninoculated); *B*, peptone Lemco agar (inoculated); *C*, gluconate medium (nutrients and agar treated; uninoculated); *D*, gluconate medium (untreated; inoculated); *E*, gluconate medium (nutrients treated; inoculated); *F*, gluconate medium (nutrients and agar treated; inoculated); *G*, gluconate medium (as *F* with addition of 0·05 per cent 'Yeastrel'; inoculated).

An ultra-violet light source with a Wood's glass filter was necessary when examining cultures as a brilliant fluorescence was not necessarily accompanied by a pigment visible to the naked eye. Neither the liquid nor solid medium showed any fluorescence without the growth of pseudomonads.

Fig. 1 shows the relative intensity of fluorescence produced in various solid media with or without the growth (48 hr. at 27°C.) of a culture of *Pseudomonas mors-prunorum* Wormald as determined by a Locarte fluorimeter (Locarte Co., 24, Emperor's Gate, London, S.W.7) standardized by a suitable aqueous solution of fluorescein. On ordinary nutrient agar this culture showed no visible fluorescent pigment even under ultra violet light and only a trace was detectable by the fluorimeter. This trace of pigment was amplified about 25 times by the use of the suggested medium (*F*) and more than 30 times when 'Yeastrel' was added at a concentration of not more than 0·05 per cent (medium *G*).

The liquid medium could be used, in addition, for the detection of keto-acid formation[3,4], a property of many *Pseudomonas*.

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Role of the Glycolytic Enzyme System in the Germination of Spores of *Bacillus coagulans* var. *thermoacidurans*

RECENT work by Halvorson and Church[1,2] has shown that the spores of *Bacillus cereus* var. *terminalis* contain a functional hexose monophosphate oxidative pathway but no Embden-Meyerhof pathway. Our studies, however, show that the spores of *Bacillus coagulans* var. *thermoacidurans* (A.T.C.C. 8038) have a complete glycolytic enzyme system which seems to play an important part in the early stages of germination.

Spores of *B. coagulans* var. *thermoacidurans* were produced at 45°C. by culturing on Difco thermoacidurans agar which was fortified with 5 p.p.m. manganese sulphate[3]. The spores were washed ten times with water by centrifugation to remove vegetative cells, and were then lyophilized. These were kept in a desiccator. It has been previously shown[4] that spores suspended in *M*/40 phosphate buffer (*p*H 7·0) could rapidly germinate with 1–5 m*M* L-alanine but not with glucose. However, when the spores were heated in the buffer for 5–10 min. at 80°C., about 50 per cent of them germinated with 10–25 m*M* glucose during 60-min. incubation at 45°C. The response of heated spores to glucose gradually increased with age. For example, the spores stored for 6 months and 18 months in the lyophilized state showed 75 and 85 per cent germination, respectively.

To understand the mechanism of germination, the effects of inhibitors on the germination of heated spores (with glucose) and unheated spores (with L-alanine) were first examined. As shown in Table 1, germination of heated spores with glucose were strongly inhibited by iodoacetate, dipyridyl, atabrine, fluoride, arsenite, and 8-hydroxyquinoline. On the other hand, the germination of unheated spores with L-alanine was not affected by most of the inhibitors except atabrine and 8-hydroxyquinoline. Since the germination of heated spores with L-alanine was also insensitive to most of the inhibitors, it is evident that the ineffectiveness of inhibitors towards the germination with L-alanine can not be attributed to impermeability to them of unheated spore cells. From the above results it appeared that the germination with glucose probably involves glycolytic breakdown of glucose whereas germination with L-alanine proceeds through some other mechanism.

It was also found that mannose, galactose, glucose-6-phosphate, fructose-6-phosphate, and fructose-1, 6-diphosphate were as effective as glucose in inducing germination of heated spores. Effectiveness of the three sugar phosphates seemed to suggest that a Embden-Meyerhof pathway was functioning during germination of our spores with glucose. Other sugars such as fructose, gluconate, 6-phosphogluconate, ribose, ribose-5-phosphate, 2-keto-gluconate, 5-keto-gluconate, and glucosamine were not effective.

In order to confirm the occurrence of glucose metabolism during germination, heated spore suspensions (10 mgm. spores per ml.) containing 20 m*M* glucose were incubated at 45°C., and samples were taken at intervals of 5–10 min. for the determination of per cent germination, glucose consumed, and lactic acid produced. Glucose was determined by Nelson's method[5], lactic acid by the method of Barker and Summerson[6], and the percentage germination by staining with malachite green and saffranin[3]. Typical results are given in Fig. 1. It is apparent that more

Table 1. EFFECTS OF INHIBITORS ON THE GERMINATION OF SPORES OF *Bacillus coagulans* VAR. *thermoacidurans* (A.T.C.C. 8038).

Inhibitors added	Per cent Inhibition*	
	Germination of unheated spores with L-alanine	Germination of heated spores with glucose
Iodoacetate, 5 m*M*	0	98
Sodium fluoride, 50 m*M*	10	70
Sodium arsenite, 5 m*M*	0	40
Atabrine, 1 m*M*	98	70
Dipyridyl, 1 m*M*	20	98
8-hydroxyquinoline, 5 m*M*	44	62

Spores were suspended in *M*/40 phosphate buffer (*p*H 7·0) with either 5 m*M* L-alanine or 20 m*M* glucose, incubated for 60 min. at 45°C.

* Per cent inhibition = $\frac{\text{Per cent germination with inhibitors}}{\text{Per cent germination without inhibitors}} \times 100$

than 70 per cent of the spores germinated during 45 min. incubation and the amount of lactic acid produced constituted 75–80 per cent of that of glucose consumed during that time of incubation. The ratio of lactic acid produced to glucose consumed further increased to 88–90 per cent when the spore suspensions were incubated in Thunberg tubes which were evacuated and filled with oxygen-free nitrogen: the rate of spore germination was not affected by anærobiosis. It should be also noted from Fig. 1 that during the incubation time from 45 min. to 90 min. the amount of lactic acid increased only slightly whereas glucose consumption remarkably continued. This fact suggests that the spores conduct glycolytic breakdown of glucose during germination, but once they germinate the germinated spores metabolize glucose through some pathway other than the typical glycolysis.

Parallel experiments using a Warburg respirometer showed that uptake of oxygen by heated spores with glucose started after 40–50 min. incubation at 45°C., and at that time most of the spores (80–90 per cent) had already germinated (Fig. 2). It can be concluded, therefore, that the germination of *B. coagulans* var. *thermoacidurans* spores with glucose depends upon glycolysis, though the outgrowth of germinated spores is accompanied by oxidative breakdown of glucose. That the terminal respiratory enzyme system develops during growth phases from spores to vegetative cells has already been shown in *Bacillus cereus*[7].

Enzymatic examinations using crude extracts of *B. coagulans* spores have revealed that the enzymes of Embden-Meyerhof pathway such as hexokinase, aldolase, triose-phosphate isomerase, and triose-

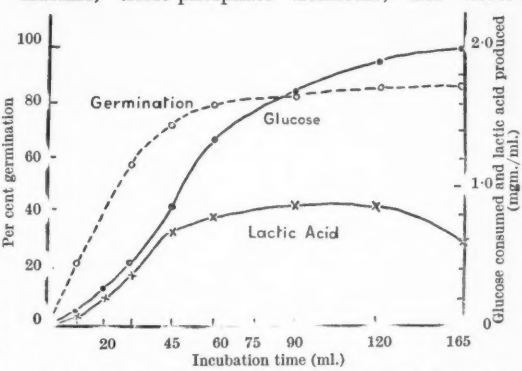

Fig. 1. Glucose utilization during germination of heated spores of *B. coagulans* var. *thermoacidurans*.

Heated spores were suspended with a concentration of 10 mgm. spores per ml. in *M*/100 phosphate buffer (*p*H 7·2) containing 20 m*M* glucose. Test tubes containing 5 ml. of the suspension were incubated at 45°C.

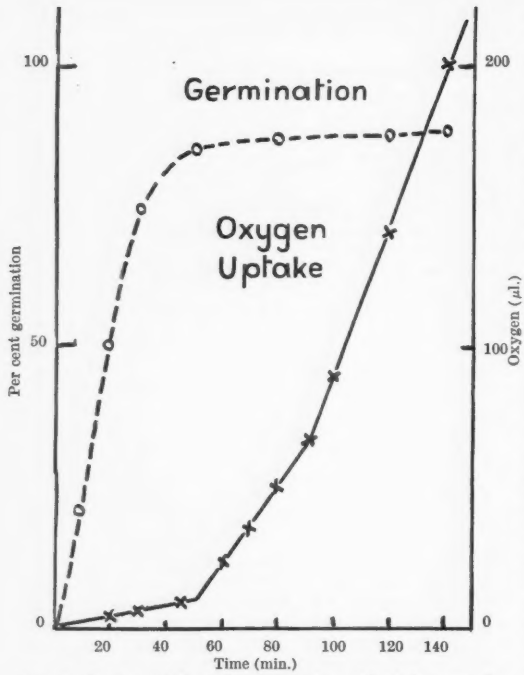

Fig. 2. Oxygen uptake during germination of heated spores of *B. coagulans* var. *thermoacidurans*. Warburg flasks contained 20 μM glucose and spores (equivalent to 10 mgm. of dry weight) suspended in 2 ml. of $M/40$ phosphate buffer (*p*H 7·0).

phosphate dehydrogenase are present in the spores.

Details of these results obtained will be published elsewhere. The work was supported by a grant from the Ministry of Education, for which we wish to express our gratitude. We also express our thanks to Dr. Z. John Ordal, University of Illinois, for his generosity in supplying the strain and some chemicals used in this study.

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Antibiotic Production as a Function of Spore Formation in *Bacillus licheniformis*

Elaboration of antibiotics by postlog-phase cells of several different micro-organisms has been emphasized recently. Conditions required for optimum production of penicillin,[1] synnematin,[2] chlortetracycline,[3] erythromycin,[4] streptomycin,[5] and neomycin[6] all provide for a fast growth period followed by a period of fermentation involving slow growth or no growth at all.

During an investigation on the biosynthesis of bacitracin by *Bacillus licheniformis*, we observed that the antibiotic is produced only after growth on glucose is complete. A microscopic examination of the culture during the time of appearance of the antibiotic indicated active spore formation, and prompted a more intensive investigation of this observation. Bacitracin is produced only under cultural conditions that support spore formation. Sporulation and antibiotic release can be inhibited by several different methods designed to prevent a rise in *p*H above 6·5, for example, glucose addition, buffer regulation, or intermittent titration. Bacitracin is not produced when sporulation is completely inhibited with ethyl malonate, whereas vegetative cell growth and *p*H are not affected. Production of this polypeptide seems to be related in some way to the spore-forming metabolism of *B. licheniformis*.

A spore suspension of *B. licheniformis*, *A*–5, was germinated by overnight incubation in a water-bath shaker in a modified medium of Hills *et al.*[7] This medium contains per litre: glucose, 3·6 gm.; ammonium lactate, 5·35 gm.; citric acid, 312·0 mgm.; crystalline magnesium sulphate, 1·0 gm.; ferrous ammonium sulphate, 25·0 mgm.; crystalline magnesium sulphate, 6·0 mgm.; sodium chloride, 400·0 mgm.; potassium chloride, 400·0 mgm.; and orthophosphoric acid, 450·0 mgm.; The *p*H of the salts mixture was adjusted to 7·4 with potassium hydroxide and sterilized independently from the carbohydrate. Growth of the cells and antibiotic production were observed in the same medium when an 8 per cent inoculum of the germinated spore culture was used. Conditions for fast growth and slow fermentation, considered optimum for the production of other antibiotics, are provided by this medium. Fig. 1 shows the time course of growth, *p*H change, bacitracin production, and spore formation. Initially, growth at the expense of glucose is rapid, with the concomitant formation of acid. The *p*H drops, but rises after the glucose has been exhausted, usually 7 hr. after inoculation. After this time, the cells begin to utilize lactic acid, and growth progresses at a much slower rate. Bacitracin production is first observed at 8 hr., and its release into the medium continues for about 20 hr. Sporulation begins in about 10 hr., with the first free spores appearing after 20 hr., and sporulation is essentially complete in 36 hr. The medium of Hills *et al.*[7] contains much higher concentration of glucose and ammonium ions, and provides for erratic and incomplete sporulation, even after 7 days.

Strange and his coworkers [8–10] and Greenberg and Halvorson[11] isolated and characterized several enzymes from sporulating cultures of *Bacillus* sp. The activity of these enzymes is directed toward cell walls and seems to be responsible for the release of polypeptides into the medium. It is interesting that the composition of bacitracin includes four D-amino-acids and ε-aspartyl-lysine residue, found in the cell

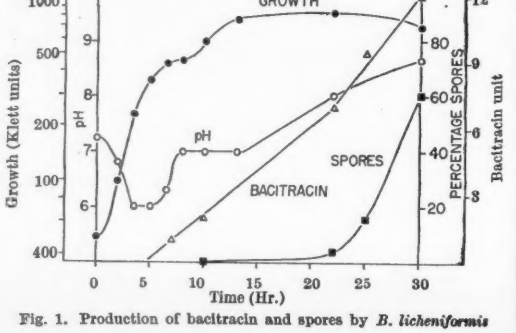

Fig. 1. Production of bacitracin and spores by *B. licheniformis*

walls of several lactobacilli, *Actinomyces bovis*, and probably in *Bacillus sphaericus*.[12]

The possibility exists, therefore, that this polypeptide antibiotic is part of the cell wall of *B. licheniformis* and is released by the activity of a lytic enzyme produced by the cell as part of its spore-forming metabolism.

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CYTOLOGY AND GENETICS

Glass Micro-Electrodes for Measuring Intracellular Activities of Sodium and Potassium

RECENTLY, Eisenman, Rudin and Casby[1] reported on the preparation of cation-sensitive glasses and indicated their potential usefulness as electrodes for biological studies. Friedman *et al.*[2,3] adapted such electrodes for measuring continuously the plasma sodium of a rabbit and dog. Caldwell[4] demonstrated that *p*H glass electrodes can be constructed on a microscale for measuring intracellular *p*H. The present report concerns construction of micro-electrodes for intracellular measurement of sodium and potassium activities.

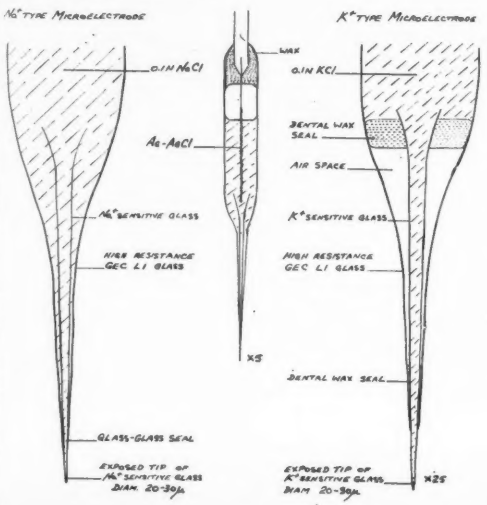

Fig. 1

Cation-sensitive glasses were supplied by Friedman and behaved similarly to Eisenman's NAS_{11-18} (sodium selective) and NAS_{27-8} (potassium selective) glass. Fig. 1 illustrates the design of the micro-electrodes. Note that a glass to glass seal was made between the *L–I* and NAS_{11-18} glass. This simplified its construction and produced a very durable and stable electrode. Since NAS_{27-8} did not fuse to *L–I*, the glasses were sealed with dental wax as shown. The trapped air prevented shorting between the inside and outside solutions when the lower wax seal broke down. No insulation other than the lead glass jacket was required. The exposed tip of cation-sensitive glass was $20\mu \times 150\mu$ with a 1–4μ wall thickness. Conventional micro-electrodes filled with $2N$ ammonium chloride were used as reference electrodes. Potential recordings were made with a 'Vibron' electrometer model 33*B* (Electronic Instruments Limited, Richmond, Surrey, England) since the glass electrode resistance ranged between 10^{10} and 10^{11} ohms.

Over the biological electrolyte range, the sodium electrode behaved according to the empirical equation[5]:

$$E = E^0 + \frac{RT}{F} \ln (a_{Na} + k_{Na-K} a_K)$$

where E, measured e.m.f.; E^0, standard potential; R, ideal gas equation constant; T, temperature (absolute); F, Faraday constant; a_{Na} and a_K, activity of sodium and potassium ions; k_{NaK}, empirical constant for a given glass composition.

Since $k_{NaK} = 0{\cdot}005$ the equation could be simplified to:

$$E = E^0 + \frac{RT}{F} \ln a_{Na}$$

with no appreciable error as long as *p*H was not less than 6·5 and a_K not greater than 0·15. The potassium electrode varied but could be calibrated daily to fit a curve:

$$E = E^0 + s \ln (a_K + k_{KNa}\, a_{Na})$$

where s = slope or mV. per unit log change. This was 3–6 mV. less than the expected 58 mV. at 20°C. E^0 also varied by ±3–5 mV. but k_{KNa} (0·125) remained relatively stable.

Muscle cells from the propodite of crab and lobster (*Carcinus mœnus* and *Homarus vulgaris*) were chosen because of their size (200–500μ diameter). The preparation consisted of a row of intact fibres mounted vertically in a bath containing (mM/l.) sodium, 513; potassium, 12·9; calcium, 11·8; magnesium, 23·6; bicarbonate, 2·6; chlorine, 594[6]. The glass electrodes were manipulated so as to pierce the membrane at an acute angle and guided along the fibre axis until the sensitive tip was about 100μ from the puncture site. The membrane potential was measured between an internal and external ammonium chloride filled capillary micro-electrode. The internal capillary was used as reference electrode in measuring the cation activity. For a chemical check, carpopodite muscles kept under similar experimental conditions were analysed for total sodium and potassium content, by flame photometry. Shaw's method of dissection was followed[7]. The intact muscle was then allowed to equilibrate for 1 hr. in the artificial bathing solution before it was rinsed for 1 min. in iso-osmotic dextrose.

Table 1 summarizes the activities calculated from six preparations (3 of the crab and 3 of the lobster, with the potassium electrodes used only in the last).

Table 1. Intracellular Activities of Sodium and Potassium of Muscle Cells

No. of Cells	Range of Membrane potential	Activity ± S.E.
	(*Carcinus mænas*)	
20	30–55 mV.	$a_{Na} = 0{\cdot}0135 \pm 0{\cdot}0008$
	(*Homaris vulgaris*)	
10	30–46 mV.	$a_{Na} = 0{\cdot}016 \pm 0{\cdot}001$
10	31–39 mV.	$a_{Na} = 0{\cdot}012 \pm 0{\cdot}0004$
12	31–52 mV.	$a_K = 0{\cdot}084 \pm 0{\cdot}0015$ $a_{Na} = 0{\cdot}015$

Table 2. Total Concentrations of Sodium and Potassium of Muscle under similar conditions as in Table 1

No. of muscles	$[Na^+] \pm S.E.$ moles/kgm. H_2O	$[K^+] \pm S.E.$ moles/kgm. H_2O
	(*Carcinus mænas*)	
12	$0{\cdot}0516 \pm 0{\cdot}0033$	$0{\cdot}169 \pm 0{\cdot}0025$
	(*Homaris vulgaris*)	
6	$0{\cdot}055 \pm 0{\cdot}0043$	$0{\cdot}153 \pm 0{\cdot}0026$

Values were accepted from cells if the membrane potential was higher than 30 mV. The standard errors are given to indicate the small variation from cell to cell despite the wide range of membrane potentials. The sodium activity is virtually the same in crab and lobster muscle. Comparison with the concentrations per litre of tissue water (Table 2) shows that concentration of sodium is at least three times greater than the measured activity of sodium, and concentration of potassium is twice as great as activity of potassium.

The experiments are of a preliminary kind and will have to be repeated under different experimental conditions and on other material. Nevertheless, they show the practicability of using these glass electrodes on a micro scale and of measuring activities of sodium and potassium in the interior of the cell.

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Production of a Perfect Stage in a Nutritionally Deficient Mutant of Pathogenic *Fusarium oxysporum* after Ultra-violet Irradiation

The genus *Fusarium* has 16 sections, many of which have a sexual stage, belonging to such Ascomycete genera as *Nectria*, *Calonectria*, *Hypomyces* and *Gibberella*. *Fusarium oxysporum*, the form species of the section *Elegans*, is a widely distributed soil-borne fungus that causes wilt in many economically important crops. It has no known perfect analogue, although it can achieve genetic variation through asexual methods of recombination[1,2].

During experimental production by ultra-violet irradiation of nutritionally deficient mutants in the pea wilt fungus, *Fusarium oxysporum* f. *pisi*, many different wild-type isolates of its physiological races have been genetically marked in this laboratory. Many such mutants retain their wild-type morphology, but others are considerably altered, usually producing more spores per amount of mycelium and, less frequently, spores of different shape from the wild-type.

One of many isolates that have been repeatedly used for artificial inoculation tests was irradiated in October 1954 and found to be deficient in its methionine and arginine synthesis. In June 1955, further irradiation of this mutant resulted in additional deficiencies in cystine and vitamin B_1 synthesis. It can grow only slowly on non-supplemented agar media.

This isolate, together with many other mutant strains, was used extensively in genetical work, which entailed repeated sub-culturing from single spores, both on a 'minimal' and 'complete' agar medium[2]. It retained its capacity to wilt peas throughout several experiments and readily formed heterokaryons with other marked strains of *F. oxysporum* f. *pisi*. It would not, however, form stable heterokaryons with isolates of *Fusarium solani* f. *pisi*, a soil-borne fungus that causes foot-rot in peas. Since March 1956, all the mutant strains have been retained by sub-culturing every 3 months on 'complete' medium.

In late 1956 the mutant strain produced a few bright red very small perithecia, which remained immature and blind, with no discernible asci. In the summer of 1957 the perithecia were again examined but still showed no sign of ascus development. After repeated examinations of the cultures, mature perithecia were finally seen in February 1959, when ascospores were observed in the abundant extruded asci.

After tentatively identifying the isolate as a *Hypomyces* sp., it was sent to the Commonwealth Mycological Institute, Kew, where Mr. C. Booth kindly identified it as *Nectria* (*Hypomyces*) *haematococca* Berk. and Br. This fungus, well known in the tropics, where it can damage citrus, cocoa and other crops, has not previously been recorded in this country. The perithecial wall is coarsely cellular and the asci are extruded from a short ostiolar neck. Each ascus contains eight two-celled hyaline ascospores which have longitudinal striæ. A fuller account of the taxonomic features will be published elsewhere.

Both micro-manipulated single ascospores and single *Fusarium*-stage conidia readily produce cultures with perithecia and *Fusarium* conidia. The *Fusarium*, which is morphologically indistinguishable from *Fusarium oxysporum* f. *pisi* and from the parent un-irradiated colonies, causes typical wilt of pea, with symptoms indistinguishable from those caused by all previous parent colonies during the past 5 years. Perithecia occur on the fungus that has been re-isolated from the reddened vascular tracts of the wilted plants. There can, therefore, be little doubt that this homothallic *Nectria* is the perfect stage of this particular wilt-inducing *Fusarium* isolate. Because of its peculiar mode of origin, it cannot yet be considered the perfect analogue of other members of the *Fusarium* section *Elegans*. Whether it arose as a direct result of ultra-violet irradiation or came indirectly from the altered nutritional needs of the mutant is an open question, but it may be that the genetic mechanism governing perithecial formation in this particular isolate was unmasked by irradiation damage to nuclear material that previously suppressed the formation of perithecia. Further work on the ability of this particular isolate to hybridize with wild-type isolates of pathogenic *F. oxysporum* should show whether it represents a more general perfect analogue of the wilt-causing *Fusaria*.

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